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# THE BENTHIC MACROFAUNA AT THE OUTFALLS OF THE UNDERWATER SEWAGE DISCHARGES IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA, ITALY)

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# **ABSTRACT**

The macrobenthic communities living at the outfalls of the five underwater sewage ducts servicing the Italian area of the Gulf of Trieste were sampled from 1990 to 1993 and analysed using uni- and multivariate techniques. 19,947 organisms from 217 taxa were identified. Polychaetes dominated the macrobenthic community, followed by molluscs; together they composed 92% of the total abundance and 80% of the number of species. Faunal composition differed between stations and was found to be influenced by sediment composition and depth rather than by discharges, even though the stations were located directly at the outfalls. Although the Biotic Index was low at all stations and no biological indicators of organic enrichment were found, the whole analysis indicated some degree of environmental unbalance, but it is similar to most soft bottom areas in the Gulf. These results indicate that the waste treatments have been effective in controlling the adverse effects of the urban discharges or at least have not negatively influenced the local benthic populations over the study period.

Key words: soft-bottoms macrobenthos, Biotic Index, sewage discharges, Gulf of Trieste, Adriatic Sea

# LA MACROFAUNA BENTONICA DELLE CONDOTTE DI SCARICO SOTTOMARINE NEL GOLFO DI TRIESTE (ALTO ADRIATICO, ITALIA)

# SINTESI

Le comunità macrobentoniche, insediate in vicinanza delle cinque condotte di scarico dei reflui nella parte italiana del Golfo di Trieste, sono state campionate dal 1990 al 1993 e sono state studiate con tecniche di analisi uni- e multivariata. Complessivamente sono stati identificati 19.947 individui appartenenti a 217 gruppi tassonomici. I policheti sono risultati il gruppo dominante, seguiti dai molluschi: i due gruppi insieme costituiscono il 92% dell'abbondanza totale e l'80% del numero di specie. La composizione faunistica di ciascuna stazione è maggiormente influenzata dalla tessitura del sedimento e dalla profondità, piuttosto che dalla vicinanza delle condotte di scarico. Sebbene i valori dell'indice biotico siano risultati bassi e non siano state ritrovate specie indicatrici di arricchimento organico, l'analisi complessiva dei popolamenti bentonici ha indicato un leggero grado di instabilità ambientale, il quale è piuttosto tipico delle comunità di fondo mobile del Golfo di Trieste. In base ai risultati ottenuti, il trattamento dei reflui scaricati dalle condotte sembra sia stato efficace e le comunità macrobentoniche non hanno mostrato evidenti alterazioni della loro struttura nel tempo, durante il periodo di studio.

Parole chiave: macrobenthos di fondo molle, indice biotico, condotte di scarico, Golfo di Trieste, Mare Adriatico

# **INTRODUCTION**

Pollution of coastal waters has been one of the big ecological concerns of the 20<sup>th</sup> century; the fast development of coastal cities, ports and tourist localities worldwide with consequent increasing concentrations of human populations, has created, among others, serious problems of disposal of urban and industrial wastes. The effects of these discharges into the sea have long been known to be harmful to the coastal zone environment, threatening the well-being of people and the lucrative benefits derived from the development of activities such as tourism, fisheries or mariculture, to cite a few.

As an alternative to direct discharges to the littoral, offshore disposal has been adopted, as far away as possible from urban centres, sometimes as direct ocean discharges (or dumping), but increasingly through underwater ducts. Those who end up in deep waters tend to be increasingly used, where possible (Diener *et al.*, 1995; Koop & Hutchings, 1996; Gallagher & Keay, 1998; Zamouri-Langar *et al.*, 2001). Nowadays, sewage treatment prior to discharge is not yet the rule worldwide, but is already normative in developed countries, especially in Europe (Urban waste water treatment directive 91/271/EEC and directive 98/15/EEC amending the former).

The assessment of ecological impacts related to sewage disposal has been documented in several types of marine communities, from soft-bottoms benthos (Ghirardelli et al., 1973; Smith et al., 1973; Pearson & Rosenberg, 1978; Otway, 1995a), to hard bottoms benthos (Littler & Murray, 1975; Fairweather, 1990; Grigg, 1994; Koop & Hutchings, 1996), and fish (Puffer et al., 1982; Grigg, 1994; Otway, 1995a, 1995b). The benthic communities are preferred as indicators of the health of marine environments, because of their main characteristics: reduced motility (i.e. incapacity of escaping polluting discharges, even if highly toxic or lethal), high diversity (i.e. selective response to environmental stress), and relatively long life cycles, which allow the observation of the short, medium, or long term effects of any discharged substance (Pearson & Rosenberg, 1978; Reish, 1980, 1986; Hily et al., 1986; Dauer, 1993; Borja et al.,

The region of the Gulf of Trieste (northern Adriatic Sea), populated for at least the last 2000 years (Stevenson *et al.*, 1999), is now heavily anthropized. In some areas, pollution of coastal waters caused by the direct discharges to sea from the riverine urban and industrial centres had caused, in the past, growing problems to the marine environment and endangered the development of the tourist "industry" (bathing, beaches) which provides the first source of income for a large part of the population (Ghirardelli *et al.*, 1973, 1975). To solve this problem, five underwater ducts connected to the waste treatment plants were built between the 70s and 80s to

serve the Italian coast of the Friuli-Venezia Giulia Region (Fig. 1).

To estimate the effects of those off-shore discharges on the environment, and in accordance with the (then) newly enforced ecological laws in the region, sampling of the macrobenthic communities was performed at the beginning of the 90s at the ducts' outfalls.

It is the purpose of this study to analyse these results trying to find the common components, which characterize the macrobenthic populations at the outfalls of the discharge ducts. In addition, these data will provide a baseline study of the conditions prevailing in the area for future reference.

# **MATERIAL AND METHODS**

# Study area description

Located at the north-eastern end of the Adriatic Sea, the Gulf of Trieste is a large and shallow embayment, with a coastline of 100 km, a surface close to 600 km<sup>2</sup> (Ogorelec et al., 1991), and a maximum depth of 25 m (Fig. 1). Average bottom salinities range from 36 to 38.5 and annual temperatures ranges are 8 to 20 °C at the bottom (Cardin & Celio, 1997). Water circulation is from southeast to northwest. Sedimentation is controlled mainly by river inputs rather than by marine currents (Brambati & Venzo, 1967); the soft bottoms are not homogeneous in composition and can vary from sands with patches of beach rocks to muds, predominantly detrital (Brambati et al., 1983). The other natural factors unique to this area that mainly influence the characteristics of the composition, evolution and persistence of its marine life are: 1) strong winds from the northeast that can provoke mixing of the waters down to the bottom; 2) thermal stratification of the water column (5-6 m at the beginning of spring until about 15 m at the end of summer) (Cardin & Celio, 1997) leading to occasional hypoxic and/or anoxic events (Aleffi et al., 1992; Orel et al., 1993; Malej & Malačič, 1995); 3) high sedimentation rates estimated at 1-2.5 mm y-1 (Ogorelec et al., 1991; Covelli & Fontolan, 1997); and 4) occasional mucilage production (Degobbis et al., 1995, 1999).

The study area is composed of the stations located directly at the outfalls of the five ducts operative in the area (Fig. 1). Stations 1 and 3 correspond to the sewage outfalls of the urban zones of the tourist cities of Lignano and Grado, characterised by considerable annual population fluctuations; e.g. Grado varied, in 1985, from 9,000 inhabitants in the winter to 53,000 in the summer, and in 2000 from 9,000 to 80,000; Lignano's population varied more drastically: in 1985, from 5,500 to 140,000 and in 2000 from 6,500 to ca. 250,000 (F.V.G., 1985, 2000). Station 2 is located at the outfall of the duct serving the Friuli lowlands, an industrial area with corresponding human settlements, with a population of about

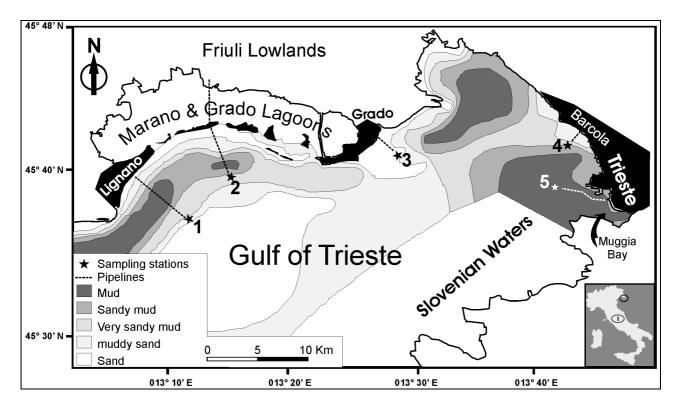


Fig. 1: Study area showing the outfalls position – sampling stations of the underwater sewage diffusers in the Italian part of the Gulf of Trieste.

Sl. 1: Raziskovano območje z vzorčevalnimi postajami ob podvodnih izlivih kanalizacijskih odplak v italijanski del Tržaškega zaliva.

Tab. 1: Main features of the five outfalls areas in the Gulf of Trieste (data from Novelli, 1996).

Tab. 1: Glavne značilnosti petih območij z vzorčevalnimi postajami ob podvodnih izpustih kanalizacijskih odplak v Tržaškem zalivu (podatki po Novelli, 1996).

| Ducts              | Treatment          | Water<br>depth (m) | Duct<br>length (m)                | Diffuser<br>length (m) | Max flow<br>(I sec <sup>-1</sup> ) |
|--------------------|--------------------|--------------------|-----------------------------------|------------------------|------------------------------------|
| Lignano (st. 1)    | secondary          | 16                 | 6000                              | 1500                   | 700                                |
| Porto Buso (st. 2) | secondary          | 15                 | 6000                              | 800+800                | 1780                               |
| Grado (st. 3)      | primary (chemical) | 10                 | 4156                              | 600                    | 456                                |
| Barcola (st. 4)    | primary            | 19                 | 900                               | 60                     | 70                                 |
| Trieste (st. 5)    | primary (chemical) | 23                 | 6000/6500<br>(two parallel ducts) | 500/1000               | 6000                               |

375,000. Station 4 serves the beach area of the city of Trieste and a residential zone (Barcola) of about 12,000 inhabitants, whereas station 5 serves Trieste, a city of about 240,000 inhabitants, with well developed industrial, commercial and port activities. All the ducts operate with diffusers at their end, Y-shaped in the case of station 2.

In Table 1, all the ducts characteristics that could be gathered are reported. It is noteworthy that no reliable data are available for the daily average flow or the nitrogen or phosphorus discharges for any of the ducts in the Gulf.

# Sampling

Sampling was performed with a 0.1 m<sup>2</sup> Van Veen grab (Aug-Sept 1990, 1991 and 1993, Nov-Dec 1990, 1991) (Fig. 1). At each station, three grabs (0.3 m<sup>2</sup>) were taken. A small fraction of each grab was preserved for sedimentological analyses. Bottom salinities and temperatures as well as oxygen concentrations were measured, using an Idronaut Mod 401 multiparametric probe.

The sediment was sieved on a 1 mm mesh and fixed in formalin following standard methodology (Holme & McIntyre, 1984), then separated and the fauna identified

to the lowest possible taxonomical level. For comparative purposes with other similar studies, the abundances were adjusted to a surface of 1 m<sup>2</sup>. Unidentified species or groups (as in the case of the amphipods), which cannot be confused with any other identified species, were included, thus the total number of taxa reported represents the lowest number of species possible. The identified species are listed in Appendix 1.

# **Analyses**

Uni- and multivariate techniques were used to analyse the communities' structure and included: abundance, number of species, diversity indexes (Shannon-Wiener diversity index (H') on loge basis (Shannon & Weaver, 1949), Margalef's index (d) (Margalef, 1958) and Pielou's evenness index (J) (Pielou, 1966)). The feeding guilds' analysis was based on Fauchald & Jumars' (1979) and Bachelet's (1981) definitions. The Bray-Curtis similarity coefficient was calculated on square-root transformed data, using complete linkage; then, non-metric multidimensional scaling ordination (MDS) (PRIMER software package developed at the Plymouth Marine Laboratory) was used to evaluate the composition similarity among the stations. Since the stress factor was found to be greater than 0.1 and following Clarke & Warwick (2001) recommendations, hierarchical classification was applied. AMBI and Biotic Index (BI) were applied (Borja et al., 2000; 2003) using the AMBI program – version 4.0 (AZTI Marine Biotic Index) (www.azti.es).

These indexes are based on the classification of the benthic species in five (I–V) ecological groups (EG), according to their tolerance to pollution (from EG–I = species very sensitive to organic enrichment, intolerant to pollution, EG–II = species indifferent to enrichment, EG-III = species tolerant to enrichment, slightly unbalanced environments, EG-IV = second-order opportunistic species, slight to pronounced unbalanced environments, to EG-V = first-order opportunistic species, pronounced unbalanced environment), then applying an algorithm to calculate the AMBI on a scale of increasing pollution (from 1 to 6) and obtaining the corresponding BI (0–1 = unpolluted sites, 2 = slightly polluted, 3 = moderately polluted, 4–5 = moderately to heavily polluted, 6 = heavily polluted, and 7 = extremely polluted, azoic state).

A recent multimetric index (M-AMBI) to assess the ecological quality status was applied, where the species richness and Shannon-Wiener diversity are also taken into consideration together with AMBI at the very same time (Muxika et al., 2007). The AMBI program provides "Bad" and "High" reference conditions as default. As "Bad" conditions the values are always 6 for AMBI and 0 for diversity and richness. For "High" conditions the software selects the lowest AMBI value and the highest diversity and richness values. The user can modify these values (bad and high) if some reference conditions have

been defined, within the Water Framework Directive, for the studied area (Muxika *et al.*, 2007). Waiting for the definition of reference conditions in Italian countries, the present paper adopted the default boundaries suggested by Borja *et al.* (2007).

## **RESULTS**

# **Abiotic parameters**

Bottom temperatures ranged from 9.34 °C (winter) to 23.55 °C (end of the summer). During the same campaign, differences among stations were negligible even if depth varied from 10 m (station 3) to 23 m (station 5) with the only exception of August 1991 when, at the deepest stations, temperature dropped of about 5 °C with respect to the shallower ones (Tab. 2). Bottom salinities varied from 36.26 to 38.20; oxygen concentrations (D.O.) values at the bottom were always around saturation, except for stations 4 and 5, at the end of the summer (Tab. 2).

Sediment composition varied from sands at station 3 to muds at station 4 and 5. At stations 1 and 2, mixed sediments were present, with higher sand content in station 1 than in station 2 (Tab. 2).

## **Faunal structure**

19,947 organisms from 217 taxa (Appendix 1) were identified; the polychaetes were by far the dominant group with 12,223 organisms (61.3% of the total population) followed by the molluscs with 6,113 organisms (30.7%). Together, they constituted 92% of the total. Crustaceans with 883 (4.4%), echinoderms with 480 (2.4%) and "others" (the remainder of the usually scarce groups such as: ascidians, anthozoans, sipunculids, nemertines, phoronids and turbellarians) with 247 organisms (1.2%), complete the list; in this last group, the sipunculids constituted 47.3%. The polychaetes were also the richest group with 125 species (57.6%), followed by the molluscs with 48 species (22.1%); together they constituted almost 80% of the total. Crustaceans (15 taxa, 6.9%), echinoderms (14 taxa, 6.5%) and "others" (15 taxa, 6.9%) followed far behind.

The number of species and the mean of Margalef index decreased from station 1 to 5 (55 to 22, 7.9 to 3.2), while abundance and H' dropped from stations 1–2 to 3, and then to 4–5. Mean values of evenness were lowest at stations 4 and 5 (Tab. 3).

Cumulative percentages of the ten most abundant species were significantly lower at stations 1–3 (55.3–65.1%) than at stations 4–5 (83.9–88.8%). Dominance of the most abundant species was more pronounced at stations 1 and 2, where it represented 6 and 7 times the percentage of the next species (Tab. 4). *Corbula gibba* was strongly dominant at stations 2, 4 and 5, but was absent at stations 1 and 3.

Tab. 2: Abiotic parameters registered at the bottom in the five ducts' outfalls of the Gulf of Trieste.

Tab. 2: Abiotični parametri, ugotovljeni na dnu petih vzorčevalnih postaj ob podvodnih izpustih odplak v Tržaškem zalivu.

| Station | Date       | depth (m) | T (°C)         | Sal   | D.O.(cm <sup>3</sup><br>dm <sup>-3</sup> ) | D.O.(%) | sand (%) | mud (%) |
|---------|------------|-----------|----------------|-------|--|---------|----------|---------|
| St. 1   | 4.9.1990   | 16.7      | 22.83          | 37.58 | 5.14                                       | 106.30  | 71.4     | 28.6    |
|         | 13.11.1990 | 15.9      | 17.79          | 36.84 | 5.20                                       | 97.64   | 75.3     | 24.7    |
|         | 29.8.1991  | 16.5      | 21.39          | 37.02 | 4.81                                       | 96.65   | 70.5     | 29.5    |
|         | 18.12.1991 | 15.7      | 9.92           | 37.03 | 5.63                                       | 90.10   | 72.2     | 27.8    |
|         | 1.9.1993   | 16.7      | 23.45          | 37.19 | 4.89                                       | 102.00  | 75.8     | 24.2    |
| St. 2   | 4.9.1990   | 16.0      | 22.86          | 37.54 | 5.04                                       | 104.26  | 42.1     | 57.9    |
|         | 13.11.1990 | 14.2      | 1 <i>7</i> .55 | 36.51 | 5.13                                       | 95.69   | 45.8     | 54.2    |
|         | 29.8.1991  | 15.4      | 23.55          | 35.91 | 4.97                                       | 103.09  | 41.2     | 58.8    |
|         | 18.12.1991 | 14.1      | 10.42          | 37.24 | 5.75                                       | 93.15   | 43.4     | 56.6    |
|         | 1.9.1993   | 15.6      | 23.45          | 37.35 | 4.47                                       | 93.33   | 42.8     | 57.2    |
| St. 3   | 4.9.1990   | 10.0      | 22.79          | 37.51 | 4.96                                       | 102.46  | 95.2     | 4.8     |
|         | 13.11.1990 | 10.4      | 1 <i>7</i> .05 | 36.42 | 5.21                                       | 96.20   | 96.9     | 3.1     |
|         | 29.8.1991  | 9.8       | 23.04          | 36.26 | 5.01                                       | 103.20  | 97.4     | 2.6     |
|         | 18.12.1991 | 8.6       | 9.34           | 36.97 | 6.14                                       | 96.98   | 96.3     | 3.7     |
|         | 1.9.1993   | 10.0      | 22.80          | 37.00 | 4.80                                       | 98.88   | 95.6     | 4.4     |
| St. 4   | 5.9.1990   | 19.5      | 21.83          | 37.96 | 1.74                                       | 35.43   | 3.8      | 96.2    |
|         | 14.11.1990 | 20.6      | 17.46          | 37.22 | 5.23                                       | 97.80   | 4.0      | 96.0    |
|         | 30.8.1991  | 19.4      | 18.41          | 37.63 | 2.83                                       | 54.02   | 3.5      | 96.5    |
|         | 19.12.1991 | 18.9      | 9.90           | 37.29 | 5.71                                       | 91.49   | 2.7      | 97.3    |
|         | 31.8.1993  | 19.7      | 22.56          | 37.68 | 3.77                                       | 77.64   | 3.3      | 96.7    |
| St. 5   | 5.9.1990   | 22.4      | 21.30          | 38.20 | 0.80                                       | 16.16   | 1.8      | 98.2    |
|         | 14.11.1990 | 21.8      | 17.52          | 37.33 | 5.11                                       | 95.73   | 1.5      | 98.5    |
|         | 30.8.1991  | 22.6      | 17.23          | 37.80 | 4.95                                       | 92.48   | 1.1      | 98.9    |
|         | 18.12.1991 | 22.3      | 10.21          | 37.38 | 5.99                                       | 96.69   | 2.0      | 98.0    |
|         | 31.8.1993  | 23.0      | 21.42          | 37.88 | 1.41                                       | 28.49   | 1.7      | 98.3    |

Tab. 3: Ecological parameters measured for this study: H' – Shannon-Wiener diversity index; d – Margalef's index; J – Pielou's evenness index.

Tab. 3: Ekološki parametri, ugotovljeni za pričujočo študijo: H' – Shannon-Wienerjev diverzitetni indeks; d – Margalefov indeks; J – Pieloujev indeks izenačenosti.

|         |     | bundan<br>(ind m <sup>-2</sup> |      | Spec | ies rich<br>(n°) | ness |     | H′  |      |     | d    |      |     | J   |      |
|---------|-----|--------------------------------|------|------|------------------|------|-----|-----|------|-----|------|------|-----|-----|------|
| Station | min | max                            | mean | min  | max              | mean | min | max | mean | min | max  | mean | min | max | mean |
| St. 1   | 710 | 1490                           | 955  | 41   | 82               | 55   | 2.5 | 3.5 | 3.1  | 5.8 | 11.1 | 7.9  | 0.7 | 0.9 | 0.8  |
| St. 2   | 170 | 1377                           | 967  | 23   | 65               | 45   | 2.0 | 3.5 | 2.7  | 4.3 | 9.1  | 6.5  | 0.5 | 8.0 | 0.7  |
| St. 3   | 343 | 1467                           | 701  | 27   | 53               | 39   | 2.6 | 3.3 | 2.9  | 4.5 | 7.7  | 5.9  | 0.7 | 0.9 | 8.0  |
| St. 4   | 363 | 1263                           | 669  | 12   | 36               | 28   | 0.9 | 2.8 | 2.1  | 1.9 | 5.4  | 4.2  | 0.4 | 8.0 | 0.6  |
| St. 5   | 490 | 847                            | 698  | 7    | 41               | 22   | 0.9 | 2.4 | 1.6  | 0.9 | 6.0  | 3.2  | 0.5 | 0.7 | 0.5  |

The dendrogram resulting from the Bray-Curtis similarity matrix (Fig. 2a) shows that the stations were more closely related among themselves than with the others, a trend confirmed by the MDS ordination (Fig. 2b); three main groups were identified: the first is formed by two subgroups constituted by stations 4 and 5, the second is formed by station 3, and the third comprises stations 1

and 2 with the only exception of the sample of 11/1990 at station 3.

The value of Biotic Index was 1 in station 1 and 2 in remaining stations (Fig. 3), which correspond respectively to unpolluted and slightly polluted conditions for the "site pollution classification", and to impoverished and unbalanced situations regarding the "benthic com-

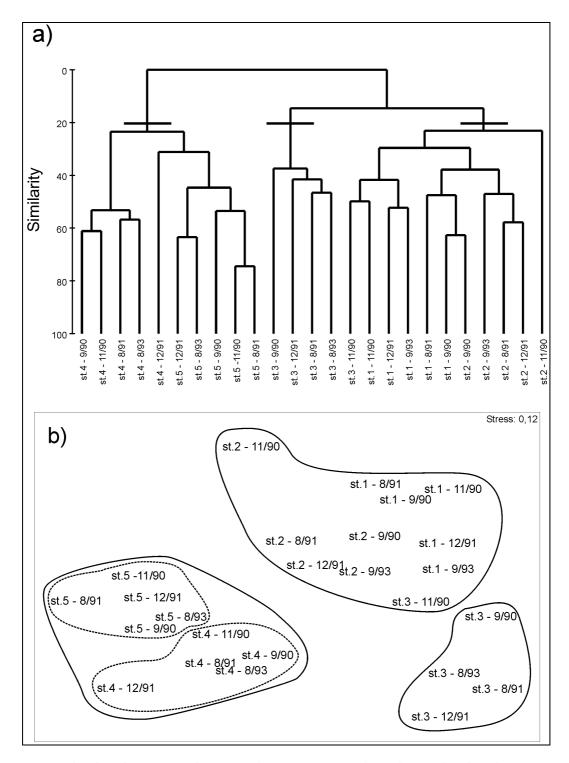


Fig. 2: (a) Hierarchical agglomerative clustering of square-root transformed macrobenthos data using complete linking on Bray-Curtis similarities (%); (b) multidimensional scaling ordination from square-root transformed macrobenthos data based on Bray-Curtis similarities.

Sl. 2: (a) Klasterski diagram na podlagi Bray-Curtisovega indeksa podobnosti; (b) večdimenzionalno skaliranje na podlagi Bray-Curtisovega indeksa podobnosti. Pri obeh analizah so bili makrobentoški podatki transformirani z uporabo kvadratnega korena.

munity health" (Borja et al., 2000). M-AMBI index revealed a "Good" to "High" quality of benthic ecological status (Fig. 4).

About feeding guilds, most dominant species were surface deposit feeders, sub-surface deposit feeders or carnivores (Tab. 4), except for the absolute dominant, *C. gibba* (ca. 23% of the total), which is a suspension-feeder.

# **DISCUSSION**

When analysing species richness in the Gulf of Trieste, we can see that the richest areas in number of species are the centre of the Gulf, around the discharges ducts of the urban centres, the zone to the east of the Isonzo estuary and the Barcola and Sistiana areas. The poorest areas were found to be in the northernmost zone, at the farther end of the Bay of Muggia and the deepest muddy areas, sometimes subjected to hypoxia events. Typical species indicating pollution conditions were found in some restricted coastal areas and in the Bay of Muggia, and generally they were in low density (Solis-Weiss et al., 2001). The average Biotic Index in the Gulf is 2 and indicates a general not polluted and quite diversified condition of macrozoobenthos, excepting some area characterized by slight or moderate disturbance due to C. gibba dominance, where both natural and man induced stresses are involved (Solis-Weiss et al., 2001; Rossin, 2005).

The Biotic Index calculated in the present study seemed to indicate a similar response of the macrofauna to the discharges, excepting the undisturbed condition revealed in station 1. However, this value is the average of very different proportions of the four ecological groups (EG) of species present at each station particularly evident at stations 1 and 5 (Fig. 3); at station 1, EG-I species clearly dominated (50%), while at station 5 there were practically no species of EG-II and III, and EG-IV species dominated, closely followed by EG-I species. In the other three stations, the proportions between ecological groups were more balanced, with a prevalence of EG-IV species in stations 2 and 4.

The application of M-AMBI (Muxika *et al.*, 2007) within the European Water Framework Directive (WFD 2000/60/EC) indicated an overall good ecological status of the study area.

The analysis of the species composition and abundance further emphasized the differences among the stations. Considering the ten most abundant species for each station (Tab. 4), *Aponuphis bilineata*, was among the first at stations 1, 2 and 3, being the dominant at station 1, while totally absent from stations 4 and 5; this species is mainly found in fine sands (Ameziane *et al.*, 1995; Desroy & Retiere, 2001). *Lucinella divaricata*, another sand dweller (Sardà *et al.*, 1999) and *Owenia fusiformis*, a species characteristic of fine sands (Pérès &

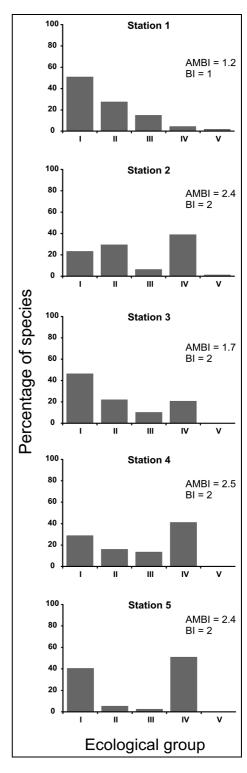


Fig. 3: Percentage of species of EG I to EG V per station with the corresponding AMBI and Biotic Index values, following Borja et al. (2003).

Sl. 3: Odstotek vrst EG I do EG V na posamezno vzorčevalno postajo z vrednostmi AMBI in biotskih indeksov po Borja et al. (2003).

Tab. 4: Cumulative percentage and feeding guilds of the first ten most abundant species in the study area: Tot. abund. – total abundance; SDF – surface deposit feeders; SSDF – sub-surface deposit feeders; C – carnivores; SF – suspension feeders.

Tab. 4: Skupni delež in prehranjevalni cehi prvih deset najštevilčnejših vrst v preučevanem območju: Tot. abund. – skupna abundanca; SDF – detritivori na površju morskega dna; SSDF – detritivori v morskem dnu; C – mesojedi organizmi; SF – filtratorji.

|                             | S    | tation 1 |             |                  |
|-----------------------------|------|----------|-------------|------------------|
| Species                     | %    | Cum %    | Tot. abund. | Feed. guilds     |
| Aponuphis bilineata         | 26.8 | 26.8     | 1280        | Č                |
| Notomastus sp.              | 4.5  | 31.3     | 217         | SDF-SSDF         |
| Chone duneri                | 4.3  | 35.7     | 207         | SF               |
| Clymene sp.                 | 4.3  | 40.0     | 207         | SSDF             |
| Maldane glebifex            | 3.7  | 43.7     | 177         | SSDF             |
| Eunice vittata              | 2.8  | 46.5     | 133         | C                |
| Laonice cirrata             | 2.4  | 48.9     | 117         | SDF              |
| Pista cristata              | 2.4  | 51.3     | 113         | SDF              |
| Chone collaris              | 2.1  | 53.4     | 100         | SF               |
| Lumbrineris latreilli       | 2.0  | 55.3     | 93          | C                |
|                             |      | tation 2 |             | -                |
| Species                     | %    | Cum %    | Tot. abund. | Feed. guilds     |
| Corbula gibba               | 35.2 | 35.2     | 1703        | SF               |
| Lumbrineris latreilli       | 5.1  | 40.3     | 247         | C                |
| Aponuphis bilineata         | 4.8  | 45.2     | 233         | C                |
| Amphiura chiajei            | 3.9  | 49.0     | 187         | SDF              |
| Lumbrineris gracilis        | 3.7  | 52.7     | 177         | C                |
| Maldane glebifex            | 2.7  | 55.4     | 130         | SSDF             |
| Clymene sp.                 | 2.6  | 58.0     | 127         | SSDF             |
| Eunice vittata              | 2.6  | 60.6     | 123         | 33DF             |
| Terebellides stroemi        |      | 63.0     | 117         | SDF              |
|                             | 2.4  | 65.1     | 103         | SF               |
| Spiochaetopterus costarum   |      |          | 103         | 3F               |
| Constitution                |      | tation 3 | Т. с        | Transfer and the |
| Species                     | %    | Cum %    | Tot. abund. | Feed. guilds     |
| Eunice vittata              | 12.2 | 12.2     | 427         | C                |
| Lucinella divaricata        | 11.2 | 23.4     | 393         | SF               |
| Clymene sp.                 | 7.4  | 30.8     | 260         | SSDF             |
| Aponuphis bilineata         | 7.3  | 38.2     | 257         | С                |
| Prionospio malmgreni        | 5.8  | 44.0     | 203         | SDF              |
| Chaetozone setosa           | 5.1  | 49.1     | 180         | SDF              |
| Poecilochaetus serpens      | 4.8  | 53.9     | 167         | SDF              |
| Prionospio caspersi         | 2.9  | 56.8     | 103         | SDF              |
| Ancistrosyllis groenlandica | 2.8  | 59.6     | 97          | С                |
| Owenia fusiformis           | 1.9  | 61.5     | 67          | SDF-SF           |
|                             |      | tation 4 |             |                  |
| Species                     | %    | Cum %    | Tot. abund. | Feed. guilds     |
| Corbula gibba               | 37.6 | 37.6     | 1257        | SF               |
| Maldane glebifex            | 10.9 | 48.5     | 363         | SSDF             |
| Melinna palmata             | 9.7  | 58.1     | 323         | SDF              |
| Eunice vittata              | 8.5  | 66.6     | 283         | С                |
| Spiochaetopterus costarum   | 6.7  | 73.3     | 223         | SF               |
| Nucula nucleus              | 4.3  | 77.6     | 143         | SDF              |
| Pectinaria koreni           | 2.2  | 79.8     | 73          | SSDF             |
| Pomatoceros triqueter       | 2.0  | 81.8     | 67          | SF               |
| Paraonis sp.                | 1.2  | 83.0     | 40          | SDF              |
| Aricidea sp.                | 1.0  | 83.9     | 33          | SDF              |
| •                           |      | tation 5 |             |                  |
| Species                     | %    | Cum %    | Tot. abund. | Feed. guilds     |
| Corbula gibba               | 47.9 | 47.9     | 1670        | SF               |
| Maldane glebifex            | 28.7 | 76.5     | 1000        | SSDF             |
| Spiochaetopterus costarum   | 2.4  | 78.9     | 83          | SF               |
| Pomatoceros triqueter       | 2.2  | 81.1     | 77          | SF               |
| Pectinaria koreni           | 1.7  | 82.8     | 60          | SSDF             |
| Nucula nucleus              | 1.4  | 84.2     | 50          | SDF              |
|                             | 1.4  |          | 47          | C                |
| Processa sp.                |      | 85.6     | 47          | SDF              |
| Terebellides stroemi        | 1.1  | 86.7     |             |                  |
| Atrina pectinata            | 1.1  | 87.9     | 40          | SF<br>SSDF       |
| Pectinaria auricoma         | 1.0  | 88.8     | 33          | 22Dt             |

Picard, 1964), were practically found only at station 3 (respectively 97.5% and 96.5% of the specimens collected in this study). Station 3 differed from the other four stations in species composition: seven out of its ten most abundant species were found as dominant only at this station (Tab. 4) and the dominances were not marked. Moreover, in contrast with the other four stations, at station 3 no echinoderms were found.

At stations 4 and 5, *C. gibba* and *Maldane glebifex* were the absolute dominants, amounting together to almost 50% and 76% of the total, respectively (Tab. 4). In addition, six out of the ten most abundant species were common to those two stations. Both *C. gibba* and *M. glebifex* are prevalently found in muddy bottoms. *C. gibba* was also strongly dominant at station 2 (35% of the total), but the faunal composition of this station was very different from the former two (Tab. 4). The changes over time of *C. gibba*'s abundance were much more evident, being very high only in the 1991 samplings. The number of total taxa was also much larger at stations 1 and 2: 138 and 105, versus 73 and 63 at stations 4 and 5.

Since three different groups (1–2, 3, 4–5) emerged as a result of uni- and multivariate analyses, these populations cannot be typified as the macrofauna "characteristic of the outfalls areas" of the Gulf.

We found that the individuated groups share similar sediment and depth conditions. Sediment composition has been found to be a key element in structuring macrofauna community (Gray, 1974), also when related to sewage outfalls. Nicolaidou *et al.* (1993), for example, found that in areas influenced by organic discharges to Saronikos Gulf (Aegean Sea), diversity increased in areas of mixed sediments, rather than related to a gradient in organic enrichment, when conditions of pollution were not too severe, *i.e.* not directly at the outfalls.

Depth is also known to influence abundance and diversity because it is highly correlated to factors such as light, temperature, hydrodynamic properties and size of sediments' particles among others (Vio *et al.*, 1980; Orel *et al.*, 1987; Diener *et al.*, 1995): in this shallow bay, even a few meters make a difference for the local benthos (Aleffi *et al.*, 1995).

The structure of each station's populations appeared firstly related to local combinations of sediment composition and secondarily to depth. These two factors seem to act synergetically in the separation of the stations groups of Figure 2. At stations 1 and 2, shallow waters and mixed sediments led to the highest values of diversity and abundance; at station 3, the shallowest and with only sandy sediments, the populations' structure was different, with the highest dominance of sand dwellers (Tab. 2); at the deepest stations 4 and 5, where muds dominated, reduced abundance and diversity were recorded. The latter are also occasionally subjected to anoxia (Orel et al., 1993).

On the other hand, even though the populations of

the five stations cannot be typified as "characteristic of the outfalls", the very strong dominance of polychaetes and molluscs in their composition (92%), and the scarcity of echinoderms constitute an indication of environmental instability or anthropic impact common to all.

However, the impact does not seem to be too severe, as indicated by the Biotic Index (Fig. 3), the species composition, the predominant ecological groups (as defined by Borja et al., 2003) and M-AMBI Index (Fig. 4). The dominant species, both in abundance and frequency (Appendix 1, Tab. 4) were C. gibba, A. bilineata, Eunice vittata and M. glebifex. C. gibba (EG-IV) is considered an indicator of instability (Orel et al., 1987; Crema et al., 1991; Elias, 1992; Aleffi & Bettoso, 2000; Zamouri-Langar et al., 2001). It is able to withstand active sediment resuspension and even hypoxia (Diaz & Rosenberg, 1995), but it is never found in conditions of severe pollution (Solis-Weiss et al., 2004). A. bilineata (EG-II) and E. vittata (EG-II) are found in unbalanced or moderately impacted bottoms, and M. glebifex (EG-I) is basically found in unpolluted muddy bottoms.

In addition, even though most abundant species were surface deposit feeders, sub-surface deposit feeders or carnivores (Tab. 4), able to withstand organically polluted conditions (Pearson & Rosenberg, 1978), no species indicating organic enrichment, or of EG-V (Borja et al., 2000), such as Capitella capitata, Malacoceros fuliginosus, Cirriformia tentaculata or even Neanthes caudata (collected only once) (Pérès & Picard, 1964; Bellan, 1967; Grassle & Grassle, 1974; Bellan & Bourcier, 1990; Cardell et al., 1999) were present. The high abundance of C. gibba at station 2 only recorded in 1991 certainly indicate a more unstable situation at that station in that period, but the source that triggered this response could not be identified.

The values reported for heavy metals related to anthropic activities (Pb, Cu, Mn, Ni, Zn, Cd and Cr) in the sediments of the study area (Rivetti, 1993; Covelli & Fontolan, 1997; Barbieri et al., 1999; Covelli et al., 2001) are difficult to evaluate as a measure of pollution, at least with reference to the local benthic populations. The fact that sediment composition importantly influences its heavy metals' concentrations (i.e. under similar conditions, in fine sediments the concentrations will always be higher) is not taken into account in Rivetti (1993) and Barbieri et al. (1999). In addition, the data published do not take into consideration the bioavailability of those metals; such studies have not been carried out so far in this area. It is very difficult to evaluate any environmental impact based only on abiotic data. Ideally, both abiotic and biotic parameters should be confronted, but we believe that the benthic populations are the best indicators of the global pollution "state" of an area, since it is their structure that most accurately reflects the synergetic effects of all substances to which they are subjected.

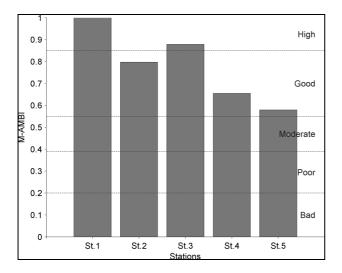


Fig. 4: M-AMBI Index per station, following Borja et al. (2007).

Sl. 4: Indeks M-AMBI na posamezno vzorčevalno postajo po Borja et al. (2007).

In this study, the values of dissolved oxygen measured at the ducts outfalls (Tab. 2) were almost always around saturation levels, *i.e.* better than expected in discharge areas.

Species composition and diversity values indicated conditions that can be qualified as "slightly polluted" or "unbalanced" (Borja *et al.*, 2000, 2003) and on the whole "Good" (Muxika *et al.*, 2007). However, in most of the other soft bottom areas of this large Gulf, similar qualifications can be applied, so that the outfalls areas come out as not particularly impacted with respect to the neighbouring zones (Solis-Weiss *et al.*, 2001).

This could mean that there is an environmental generalised impact or stress in the whole Gulf. It has been reported that the northern Adriatic benthic macrofauna has a lower number of species but higher values of abundance and biomass than the southern region (Gamulin-Brida, 1967; Šimunović, 1997) thus reflecting "harsher" environmental conditions. The Gulf of Trieste represents the most extreme conditions since it is located at the northeastern end of the Adriatic and under strong natural stress factors already outlined in the study area section, to which the anthropic pressure is added. Consequently "unbalanced" conditions are generalized throughout the area, with the influence of the natural stress factors prevailing over the anthropic pressure. It is noteworthy that at all stations diversity was higher than expected at an outfall zone (Bellan & Bourcier, 1990; Tsutsumi, 1990; Méndez, 1993; Taylor *et al.*, 1998), particularly at stations 1 and 2 where benthic macrofauna is among the richest and more diversified in the entire Gulf (Solis-Weiss *et al.*, 2001).

One possible explanation that should be verified could be that in these oligotrophic-mesotrophic waters of the northern Adriatic (Fonda Umani, 1996), some organic matter input could favour the local benthic populations. Several authors (Pearson & Rosenberg, 1978; Wilkinson, 1999) have indicated that in conditions of moderate organic enrichment, an increase in the number of species can occur, before an eventual decline.

# **CONCLUSIONS**

Since little was known about the soft bottoms macrobenthos surrounding the sewage discharge areas in the Gulf of Trieste, the analysis of these first samplings open new insights on the real effect of the local discharges on the benthic fauna, although at this point they are to be considered as preliminary results and the baseline for further studies. The macrobenthic populations' structure differed between stations and was found to be more influenced by local environmental parameters, such as sediment composition and depth, than by the selected common denominator: *i.e.* the sewage discharges.

It is imperative to continue these studies and establish a monitoring programme to evaluate accurately the ecological importance of the impacts in the medium and long range, and (hopefully) confirm these trends over the following years. Within this framework, the heavy metals' bio-availability should be assessed, in order to interpret correctly the source and the effects of the impacts on the local marine fauna, since so far, these results potentially indicate that the waste treatments have been effective in controlling the negative effects of urban discharges on those bottoms or at least have not negatively influenced their populations.

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# BENTOŠKA MAKROFAVNA PRI PODVODNIH IZPUSTIH KANALIZACIJSKIH ODPLAK V TRŽAŠKI ZALIV (SEVERNO JADRANSKO MORJE, ITALIJA)

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## **POVZETEK**

Med letoma 1990 in 1993 so avtorji vzorčevali in z uporabo uni- in multivariatnih metod analizirali makrobentoške združbe, živeče pri izlivih odplak, ki se po petih podvodnih kanalizacijskih ceveh stekajo v Tržaški zaliv. Ugotovljenih je bilo 19.947 organizmov, pripadajočih 217 taksonom. V makrobentoški združbi so prevladovali mnogoščetinci in mehkužci, saj so sestavljali 92% celotne abundance in 80% števila vseh zabeleženih vrst. Ugotovljeno je bilo, da obstajajo razlike v favnistični sestavi na posameznih vzorčnih postajah in da nanje vplivata bolj sestava usedlin in globina kot pa odplake same, četudi je vzorčenje potekalo neposredno ob njihovih izlivih v Tržaški zaliv. Čeprav je bil biotski indeks nizek na vseh postajah in avtorji niso našli nobenih kazalcev organske obogatitve, je celotna analiza pokazala na določeno mero okoljskega neravnotežja, ki je sicer podobno neravnotežju v večini območij Tržaškega zaliva z mehkim dnom. Te ugotovitve kažejo, da je bilo čiščenje odplak učinkovito pri nadzorovanju škodljivih vplivov urbanih odpadnih voda ali pa da te vsaj niso negativno vplivale na lokalne bentoške populacije med preučevanim obdobjem.

Ključne besede: makrobentos mehkega dna, biotski indeks, kanalizacijske odplake, Tržaški zaliv, Jadransko morje

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# APPENDIX / DODATEK

List of the macrofauna species found in the study area with total abundance and frequency as presences of species in the 25 collected samples.

Seznam makrofavnističnih vrst, odkritih v preučevanem območju s skupno številčnostjo in frekvenco pojavljanja vrst v petindvajsetih nabranih vzorcih.

| Species                     | ind. m <sup>-2</sup> | freq.  |
|-----------------------------|----------------------|--------|
| Annelida                    |                      | -      |
| Amage adspersa              | 3                    | 1      |
| Ampharete acutifrons        | 17                   | 4      |
| Amphicteis gunneri          | 10                   | 1      |
| Amphitrite variabilis       | 7                    | 2<br>7 |
| Ancistrosyllis groenlandica | 117                  | 7      |
| Aonides oxycephala          | 33                   | 3      |
| Aponuphis bilineata         | 1773                 | 16     |
| Arabella geniculata         | 33                   | 5      |
| Aricidea sp.                | 190                  | 16     |
| Capitellidae indet.         | 150                  | 8      |
| Ceratonereis costae         | 7                    | 2      |
| Chaetopterus variopedatus   | 10                   | 2      |
| Chaetozone setosa           | 193                  | 5      |
| Chone acustica              | 43                   | 4      |
| Chone collaris              | 177                  | 5      |
| Chone duneri                | 253                  | 9      |
| Cirratulidae indet.         | 297                  | 17     |
| Cirratulus filiformis       | 7                    | 1      |
| Cirriformia tentaculata     | 3                    | 1      |
| Clymene sp.                 | 593                  | 15     |
| Clymenura clypeata          | 13                   | 2      |
| Dasybranchus caducus        | 23                   | 3      |
| Dorvillea sp.               | 3                    | 1      |
| Drilonereis filum           | 57                   | 6      |
| Euchone rosea               | 17                   | 2      |
| Euclymene lumbricoides      | 10                   | 3      |
| Euclymene oerstedi          | 60                   | 6      |
| Euclymene palermitana       | 103                  | 5      |
| Eulalia sp.                 | 13                   | 2      |
| Eunereis longissima         | 3                    | 1      |
| Eunice vittata              | 993                  | 24     |

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| 5 1 1 1                       | 1.0  | 0  |
|-------------------------------|------|----|
| Eupolymnia nebulosa           | 10   | 3  |
| Eupolymnia nesidensis         | 10   | 3  |
| Glycera alba                  | 3    | 1  |
| Glycera capitata              | 3    | 1  |
| Glycera rouxii                | 127  | 13 |
| Glycera sp.                   | 30   | 4  |
| Glycera unicornis             | 40   | 8  |
| Goniada maculata              | 73   | 6  |
| Harmothoe extenuata           | 3    | 1  |
| Harmothoe sp.                 | 10   | 2  |
| Jasmineira caudata            | 3    | 1  |
| Jasmineira elegans            | 60   | 4  |
| Laonice cirrata               | 197  | 12 |
| Lumbrineris gracilis          | 340  | 15 |
| Lumbrineris latreilli         | 397  | 19 |
| Lumbrineris tetraura          | 20   | 3  |
| Magelona alleni               | 13   | 4  |
| Malacoceros sp.               | 7    | 2  |
| Maldane glebifex              | 1673 | 19 |
| Marphysa bellii               | 60   | 10 |
| Marphysa sanguinea            | 20   | 6  |
| Megalomma vesiculosum         | 30   | 5  |
| Melinna palmata               | 380  | 12 |
| Monticellina dorsobranchialis | 43   | 6  |
| Myriochele heeri              | 70   | 1  |
| Myriochele oculata            | 67   | 6  |
| Mysta picta                   | 53   | 9  |
| Myxicola infundibulum         | 3    | 1  |
| Nainereis laevigata           | 3    | 1  |
| Neanthes caudata              | 10   | 1  |
| Nematonereis unicornis        | 3    | 1  |
| Nephthys hombergi             | 3    | 1  |
| Nephthys hystricis            | 3    | 1  |
| Nereis lamellosa              | 77   | 11 |
| Nereis rava                   | 23   | 3  |
| Nereis sp.                    | 63   | 8  |
| Nicomache sp.                 | 3    | 1  |
| Nothria conchylega            | 27   | 4  |
| Notomastus latericeus         | 30   | 3  |
| Notomastus sp.                | 347  | 14 |
| Ophiodromus flexuosus         | 7    | 2  |
| Orbinia cuvieri               | 7    | 1  |
| Owenia fusiformis             | 193  | 13 |
|                               | -    |    |

| Paraonis sp.                          | 53      | 7        |
|---------------------------------------|---------|----------|
| Pectinaria auricoma                   | 60      | 7        |
| Pectinaria auricoma Pectinaria koreni | 170     | 11       |
| Petaloproctus terricola               | 23      | 6        |
| Pherusa monilifera                    | 10      | 3        |
| Pherusa plumosa                       | 7       | 2        |
| Phyllodoce laminosa                   |         | 4        |
| Phyllodoce lineata                    | 40      | 7        |
| Phyllodoce mucosa                     | 3       | 1        |
| Phyllodoce sp.                        | 23      | 3        |
| Phyllodocidae indet.                  | 13      | 1        |
| Phylo foetida                         | 10      | 3        |
| Pilargis verrucosa                    | 3       | 1        |
|                                       | <u></u> | 1        |
| Piromis eruca                         | · ·     | 4        |
| Pista cristata                        | 183     | 1        |
| Platynereis dumerilii                 | 3       | ·        |
| Poecilochaetus serpens                | 233     | 16       |
| Polydora ciliata                      | 27      | 4        |
| Polydora flava                        | 37      | 7        |
| Polynoidae indet.                     | 27      | 6        |
| Pomatoceros triqueter                 | 173     | 10       |
| Prionospio caspersi                   | 103     | 1        |
| Prionospio cirrifera                  | 27      | 3        |
| Prionospio malmgreni                  | 207     | 4        |
| Pseudopolydora antennata              | 23      | 5        |
| Pseudopotamilla reniformis            | 7       | 2        |
| Sabellidae indet.                     | 23      | 4        |
| Sabellides octocirrata                | 3       | 1        |
| Scalibregma inflatum                  | 10      | 1        |
| Schistomeringos rudolphii             | 7       | 2        |
| Scolaricia typica                     | 10      | 1        |
| Scolelepis cantabra                   | 3       | 1        |
| Scolelepis tridentata                 | 7       | 2        |
| Scoloplos armiger                     | 7       | 2        |
| Serpula concharum                     | 3       | 1        |
| Serpula vermicularis                  | 20      | 2        |
| Sosane sulcata                        | 7       | 2        |
| Spio filicornis                       | 50      | 6        |
| Spiochaetopterus costarum             | 457     | 17       |
| Spionidae indet.                      | 13      | 1        |
| Spiophanes kroyeri                    | 33      | 7        |
| Sternaspis scutata                    | 10      | 3        |
| Sthenelais boa                        | 43      | 8        |
| Sthenelais minor                      | 3       | 1        |
| Sthenolepis hyleni                    | 10      | 2        |
| Streblosoma bairdi                    | 10      | 2        |
| Syllis cornuta                        | 13      | 3        |
| Syllis sp.                            | 3       | 1        |
| Terebella lapidaria                   | 7       | 1        |
| Terebellidae indet.                   | 60      | 3        |
| Terebellides stroemi                  | 160     | 6        |
| Mollusca                              |         |          |
| Abra alba                             | 33      | 6        |
|                                       |         | <u> </u> |

|                            | 1    |    |
|----------------------------|------|----|
| Abra prismatica            | 13   | 1  |
| Acanthocardia aculeata     | 7    | 2  |
| Acanthocardia paucicostata | 3    | 1  |
| Acanthochitona aenea       | 7    | 2  |
| Anodontia fragilis         | 77   | 11 |
| Anomia ephippium           | 7    | 1  |
| Atrina pectinata           | 40   | 4  |
| Azorinus chamasolen        | 10   | 3  |
| Callista chione            | 3    | 1  |
| Calyptraea chinensis       | 7    | 2  |
| Chamelea gallina           | 3    | 1  |
| Chlamys varia              | 7    | 1  |
| Corbula gibba              | 4667 | 19 |
| Dentalium inaequicostatum  | 20   | 4  |
| Diodora gibberula          | 3    | 1  |
| Diplodonta rotundata       | 37   | 4  |
| Dosinia lupinus            | 7    | 2  |
| Eulima glabra              | 3    | 1  |
| Euspira guillemini         | 3    | 1  |
| Euspira nitida             | 10   | 3  |
| Glycymeris insubrica       | 3    | 1  |
| Hiatella arctica           | 17   | 4  |
| Lima exilis                | 3    | 1  |
| Limea loscombi             | 3    | 1  |
| Loripes lacteus            | 30   | 6  |
| Lucinella divaricata       | 403  | 7  |
| Modiolarca subpicta        | 7    | 2  |
| Mysia undata               | 3    | 1  |
| Nucula nucleus             | 227  | 12 |
| Ostrea edulis              | 7    | 2  |
| Paphia aurea               | 37   | 6  |
| Pecten jacobaeus           | 3    | 1  |
| Phaxas adriaticus          | 60   | 10 |
| Philine aperta             | 20   | 3  |
| Pitar rudis                | 53   | 7  |
| Plagiocardium papillosum   | 7    | 1  |
| Proteopecten glaber        | 3    | 1  |
| Psammobia fervensis        | 20   | 5  |
| Scapharca inaequivalvis    | 3    | 1  |
| Solecurtus strigilatus     | 3    | 1  |
| Spisula subtruncata        | 30   | 3  |
| Śtriarca lactea            | 3    | 1  |
| Tellina distorta           | 113  | 12 |
| Tellina serrata            | 7    | 1  |
| Thracia convexa            | 3    | 1  |
| Thracia pubescens          | 23   | 4  |
| Thyasira flexuosa          | 53   | 6  |
| Crustacea                  |      |    |
| Amphipoda indet.           | 493  | 16 |
| Corystes cassivelaunus     | 10   | 3  |
| Ethusa mascarone           | 27   | 5  |
| Galathea intermedia        | 3    | 1  |
| Isopoda indet.             | 120  | 11 |
|                            |      |    |

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| Macropipus vernalis            | 3   | 1  |
|--------------------------------|-----|----|
| Ostracoda indet.               | 3   | 1  |
| Paguridea indet.               | 10  | 2  |
| Paguristes eremita             | 10  | 2  |
| Pilumnus hirtellus             | 7   | 2  |
| Pisidia bluteli                | 43  | 8  |
| Pisidia sp.                    | 3   | 1  |
| Processa edulis                | 10  | 2  |
| Processa parva                 | 17  | 1  |
| Processa sp.                   | 123 | 13 |
| Echinodermata                  |     |    |
| Amphiura chiajei               | 250 | 11 |
| Astropecten aranciacus         | 10  | 3  |
| Astropecten sp.                | 7   | 2  |
| Cucumaria planci               | 3   | 1  |
| Holoturidea indet.             | 3   | 1  |
| Ophiothrix quinquemaculata     | 23  | 4  |
| Ophiura albida                 | 67  | 9  |
| Ophiura grubei                 | 13  | 1  |
| Ophiura texturata              | 20  | 5  |
| Paracentrotus lividus          | 10  | 2  |
| Psammechinus microtuberculatus | 17  | 5  |
| Schizaster canaliferus         | 27  | 5  |
| Thyone fusus                   | 27  | 2  |
| Trachythyone elongata          | 3   | 1  |

| Others                    |       |   |
|---------------------------|-------|---|
| Anthozoa indet.           | 10    | 3 |
| Ascidiacea indet.         | 3     | 1 |
| Ascidiella aspersa        | 7     | 1 |
| Aspidosiphon muelleri     | 7     | 1 |
| Branchiostoma lanceolatum | 40    | 4 |
| Calliactis parasitica     | 3     | 1 |
| Edwardsia claparedi       | 3     | 1 |
| Golfingia sp.             | 7     | 1 |
| Golfingia vulgare         | 13    | 1 |
| Nemertea indet.           | 13    | 3 |
| Phascolosoma sp.          | 7     | 1 |
| Phoronida indet.          | 40    | 4 |
| Sipuncula indet.          | 53    | 7 |
| Sipunculus nudus          | 30    | 4 |
| Turbellaria indet.        | 10    | 3 |
| TOTAL ABUNDANCE           | 19947 |   |
| TOTAL TAXA                | 217   |   |

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# DIET OF THE MARBLED ELECTRIC RAY *TORPEDO MARMORATA* (CHONDRICHTHYES: TORPEDINIDAE) OFF THE LANGUEDOCIAN COAST (SOUTHERN FRANCE, NORTHERN MEDITERRANEAN)

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# **ABSTRACT**

A study of stomach contents of the electric marbled ray Torpedo marmorata Risso, 1810 has shown that the species is a rather active feeder, consuming mostly teleosts and, occasionally, cephalopods. This confirms the fact that T. marmorata is an ichthyophagous species, such as specimens from other areas and other species of the genus Torpedo.

Key words: Chondrichthyes, Torpedo marmorata, diet, coast of Languedoc, Mediterranean Sea

# ALIMENTAZIONE DELLA TORPEDINE MAREZZATA *TORPEDO MARMORATA* (CHONDRICHTHYES: TORPEDINIDAE) AL LARGO DELLA COSTA DI LANGUEDOC (FRANCIA MERIDIONALE, MEDITERRANEO SETTENTRIONALE)

# SINTESI

Lo studio dei contenuti stomacali della torpedine marezzata Torpedo marmorata Risso, 1810 ha evidenziato che la specie si alimenta attivamente, consumando in prevalenza teleostei e occasionalmente cefalopodi. I dati confermano che T. marmorata è una specie ittiofaga, come precedentemente riscontrato su individui provenienti da altre aree e su altre specie del genere Torpedo.

Parole chiave: Chondrichthyes, Torpedo marmorata, alimentazione, costa di Languedoc, Mediterraneo

Christian CAPAPÉ et al.: DIET OF THE MARBLED ELECTRIC RAY TORPEDO MARMORATA (CHONDRICHTHYES: TORPEDINIDAE) ..., 17–22

## **INTRODUCTION**

The marbled electric ray Torpedo marmorata Risso, 1810 is known in the eastern Atlantic from the northern areas such as off Scandinavia (Muus & Dahlstrøm, 1964-1966) and off the British Isles (Wheeler, 1969) to the Gulf of Guinea (Blache et al., 1970). Smith & Heemstra (1986) reported the species off South Africa, but this occurrence in the area needs confirmation. T. marmorata is reported throughout the Mediterranean, but it is rather abundantly reported in the western than in the eastern basin, and off the northern than off the southern shore (Capapé, 1989). The reproductive biology had previously been studied for specimens from the eastern Atlantic, north of the Strait of Gibraltar (France; see Mellinger, 1969, 1971, 1973, 1974, 1976), south of the Strait of Gibraltar (Senegal; see Capapé et al., 2001), and off Tunisia (Capapé, 1979). In contrast, little has been studied regarding the diet and feeding habits of the marbled electric ray, which is known as a piscivorous species (Belbenoît, 1970; Belbenoît & Bauer, 1972). Additionally, Capapé (1979) reported observations on stomach contents of specimens from Tunisian coastal waters. Off the coast of Languedoc, T. marmorata had formerly been reported by Doumet (1860), Moreau (1881), Calvet (1905), Euzet (1960) and Quignard et al. (1962), and the species is the most common torpedinid recorded in the area (Capapé et al., 2006). Observations based on several specimens caught in the area allow us to present herewith data on diet composition of the Languedocian electric marbled rays.

## **MATERIAL AND METHODS**

A total of 102 specimens, 43 males and 59 females, were examined. The observed specimens were collected off the Languedocian coast (Fig. 1) by demersal gill-nets, at depths between 10 and 50 m, on sandy and muddy bottoms, between 2001 and 2004. Total length (TL) of all the specimens was measured to the nearest millimetre; they were weighed to the nearest gram. Males ranged from 230 to 320 mm TL and weighed from 270 to 550 g, while females ranged from 195 to 550 mm TL and weighed from 200 to 2940 g.

As soon as they were collected, the electric marbled rays were dissected and the stomach contents removed, sorted and identified to the lowest taxon (species level when possible) using key and fields guides (Riedl, 1963; Fischer et al., 1981, 1987). The prey items were counted and weighed to the nearest decigram after removal of surface water by blotting on tissue paper. To analyse the diet composition of Torpedo marmorata, we used some indices following Berg (1979), Hyslop (1980) and Tirasin & Jörgensen (1999): vacuity index (VI) = number of empty stomachs divided by the total number of stomachs; percentage frequency of occurrence (% F) = the number of stomachs, in which a food item was found expressed as percentage of the total number of stomachs; percentage numerical abundance (% Cn) = number of each prey type, expressed as a percentage of the total number of all food types in all stomachs; percentage ponderal composition (% Cw) = wet weight of each

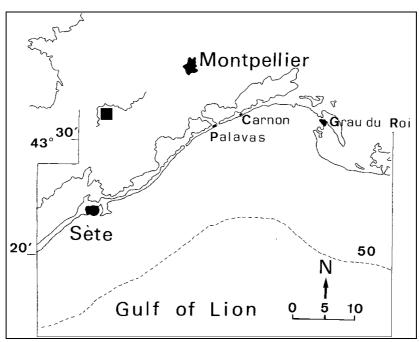


Fig. 1: Map of France with the coast of Languedoc (redrawn from Capapé et al., 2000). Sl. 1: Zemljevid Francije z obrežjem pokrajine Languedoc (po Capapé et al., 2000).

prey type, expressed as a percentage of the total weights of stomach contents in a sample.

Additionally, we used the index of relative abundance, IRI (Pinkas *et al.*, 1971; Cortès, 1997) as IRI = % F × (% Cn = % Cw), expressed as a percentage to quantify the diet as % IRI = (IRI /  $\Sigma$  IRI) × 100.

## **RESULTS AND DISCUSSION**

Of the 102 stomach contents of *Torpedo marmorata* examined, 63 were empty (VI = 61.76). Significant differences were recorded between sexes, VI were 46.51 and 72.88 for males and females, respectively ( $\chi^2_{=}$  23.5, df = 1).

The diet of the marbled electric ray consisted of two major systematic groups, cephalopods and teleosts, however, six species were ingested only (Tab. 1): the European squid *Loligo vulgaris* Lamarck, 1798, the elegant cuttlefish *Sepia elegans* Blainville, 1827, the dragonet *Callionymus lyra* Linnaeus, 1758, the red bandfish *Cepola rubescens* Linnaeus, 1766 and the leaping grey mullet *Liza saliens* (Risso, 1810), which was the most identifiable prey species (IRI = 281.88).

The vacuity index calculated for the Languedocian *T. marmorata* is lower than this observed for the Tunisian specimens. The latter specimens were captured thirty years ago, between 1970 and 1975 (Capapé, 1979); formerly, the Tunisian coast was not the focus of an intensive fishery during this period, consequently the biological environment was relatively rich (Lubet & Azouz, 1969). Specimens from Languedocian waters have more recently been examined in the second area that is intensively exploited by both commercial and craft fisheries. Fishing method could also explain differences in the va-

cuity index recorded between the two areas. Off the Languedocian coast, the marbled electric rays from our sample were caught by gill-nets, in which they spent all night and thus could not seek their prey; additionally, the prey consumed prior to the predators being caught could be entirely digested. A great number of both cephalopod and teleost prey items remained unidentified (see Table 1).

In contrast, off the Tunisian coast, they were caught by trawl, and they could accidentally ingest prey during capture. This could also explain why more teleost species were identified in Tunisian T. marmorata than in the Languedocian specimens, 13 vs. 3. Additionally, teleosts species constituted quantitatively and qualitatively the main prey species T. marmorata from both areas, whilst cephalopods were occasionally preyed, a single camarote shrimp Penaeus kerathurus (Forskäl, 1764) was accidentally found in the stomach of a Tunisian specimen. Similar patterns were observed in the diet of T. marmorata from off the coast of Senegal (unpubl. data), as well as in the diet of its close relative species, the common torpedo Torpedo torpedo (Linnaeus, 1758) from off the Tunisian coast (Quignard & Capapé, 1974). T. marmorata is a selective predator that feeds quasi exclusively on teleost species. This is due to the prev capture behaviour and feeding habits of the species of the genus Torpedo. Belbenoit & Bauer (1972) first videorecorded and described T. marmorata capturing its prey in captivity. Generally, T. marmorata at rest was waiting for prey while hidden in the sand. So, when a prey swims near a marbled electric ray at a short distance, 40 mm approximately, the latter jumps and simultaneously immobilizes the prey by its electric organ discharge. Prior to jumping, the predator detects the prey with its receptor

Tab. 1: Diet composition of the 102 marbled electric rays collected off the Languedocian coast. Legend: % F – frequency of occurrence; % Cn – percentage numerical composition; % Cw – percentage ponderal composition; IRI – index of relative importance.

Tab. 1: Sestava hrane v želodcih 102 navadnih električnih skatov, ujetih v obrežnih vodah pokrajine Languedoc. Legenda: % F – frekvenca pojavljanja; % Cn – številčnost v odstotkih; % Cw – biomasa v odstotkih; IRI – indeks relativne pomembnosti.

| Food items         | (% F) | (% Cn) | (% Cw) | IRI     | % IRI |
|--------------------|-------|--------|--------|---------|-------|
| Cephalopods        |       |        |        |         |       |
| Loligo vulgaris    | 0.98  | 2.00   | 0.70   | 2.64    | 0.06  |
| Sepia elegans      | 0.98  | 2.00   | 3.68   | 5.57    | 0.12  |
| Unidentified preys | 2.94  | 6.00   | 0.69   | 19.67   | 0.43  |
| Total              | 4.90  | 10.25  | 5.07   | 27.88   | 0.61  |
| Teleosts           |       |        |        |         |       |
| Callionymus lyra   | 0.98  | 2.00   | 1.32   | 3.25    | 0.07  |
| Cepola rubescens   | 1.96  | 4.00   | 7.24   | 23.03   | 0.59  |
| Liza saliens       | 1.96  | 4.00   | 43.75  | 93.59   | 2.05  |
| Unidentified       | 37.25 | 76.00  | 42.53  | 4415.24 | 96.68 |
| Total              | 42.15 | 86.00  | 94.84  | 4653.95 | 99.39 |

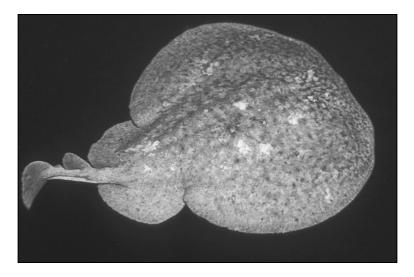


Fig. 2 / Sl. 2: Torpedo marmorata. (Photo / Foto: B. Furlan)

organs. The discharge is sufficient to break the vertebral column of the prey, which moves under the disc and is then absorbed. Active swimmers, such as teleosts, produce flue water that induces electric discharges. This explains their importance in the diet of *T. marmorata*, and offers us an opportunity to state, in agreement with previous papers, that the marbled electric ray is a piscivorous species (Fig. 2).

The diet composition of *T. marmorata* from both Tunisian and Languedocian coasts has shown that the spe-

cies feeds on more species than its close relative *T. torpedo* from the same area (Quignard & Capapé, 1974; Capapé, 1979). According to Capapé (1979), this phenomenon could be due to the fact that *T. marmorata* reaches a larger size than *T. torpedo* and is, consequently, a more active feeder. Similar patterns were reported in other elasmobranch species, such as rajids (Capapé & Azouz, 1976; Capapé, 1977a; Capapé & Quignard, 1977) and myliobatids (Capapé, 1976, 1977b; Jardas *et al.*, 2004).

# PREHRANA NAVADNEGA ELEKTRIČNEGA SKATA *TORPEDO MARMORATA* (CHONDRICHTHYES: TORPEDINIDAE) V OBREŽNIH VODAH POKRAJINE LANGUEDOC (JUŽNA FRANCIJA, SEVERNO SREDOZEMSKO MORJE)

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## **POVZETEK**

Analiza hrane v želodcih navadnega električnega skata Torpedo marmorata Risso, 1810 je pokazala, da se ta vrsta prehranjuje zelo aktivno, predvsem s pravimi kostnicami, občasno pa tudi z glavonožci. To potrjuje že znano dejstvo, da je T. marmorata ribojeda vrsta, tako kot osebki iz drugih območij in drugih vrst iz rodu Torpedo.

Ključne besede: Chondrichthyes, Torpedo marmorata, prehrana, obrežne vode Languedoca, Sredozemsko morje

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# INCIDENTAL CAPTURES OF THRESHER SHARKS (LAMNIFORMES: ALOPIIDAE) FROM TURKISH COASTAL WATERS

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# **ABSTRACT**

Twenty-one alopiid sharks, 2 bigeye thresher (Alopias superciliosus) and 19 common thresher sharks (Alopias vulpinus), were incidentally captured in the coastal waters of Turkey. General information on the captured specimens is reported and the current status of conservation of alopiids is discussed.

Key words: Alopiidae, Alopias superciliosus, Alopias vulpinus, small-scale fishery, conservation, Turkish waters

# CATTURE ACCIDENTALI DI SQUALI ALOPIDI (LAMNIFORMES: ALOPIIDAE) IN ACQUE COSTIERE TURCHE

# SINTESI

Ventuno esemplari di squali alopidi, due di Squalo Volpe Occhione (Alopias superciliosus) e 19 di Squalo Volpe Comune (Alopias vulpinus), sono stati catturati accidentalmente nelle acque costiere della Turchia. Nell'articolo vengono fornite le informazioni generali degli esemplari catturati e viene discusso lo stato di conservazione attuale degli alopidi.

**Parole chiave:** Alopiidae, *Alopias superciliosus, Alopias vulpinus,* pesca su piccola scala, conservazione, acque della Turchia

## **INTRODUCTION**

Thresher sharks (Lamniformes: Alopiidae) are large, active, strong-swimming sharks, ranging in habitat from coastal to epipelagic and deepwater epibenthic, and distributed worldwide in tropical, subtropical and coldtemperate waters (Compagno, 1984). Until now, two species, Alopias superciliosus (Lowe, 1839) and Alopias vulpinus (Bonnaterre, 1788), have been recorded in the Mediterranean and adjacent waters (De Maddalena & Baensch, 2005; Serena, 2005). The first record of A. superciliosus in the Mediterranean Sea was reported by Cigala Fulgosi (1983), based on four specimens captured by the fishermen of Mazara del Vallo (Trapani) in the Sicilian Channel. According to Serena (2005), the bigeyed thresher shark is an occasional/rarely captured species in Mediterranean waters. Let us add that De Maddalena & Baensch (2005) reported A. superciliosus as being relatively common in the Mediterranean Sea, and that both Barrull & Mate (2002) and De Maddalena & Baensch (2005) described its presence in the Mediterranean as stable, and not occasional. On the other hand, A. vulpinus is fairly common in the Mediterranean Sea (De Maddalena & Baensch, 2005; Serena, 2005); many recordings of thresher shark have been made both by general ichthyological or faunistic works (e.g. Riedl, 1983; Quéro, 1984), and regional ichthyological or shark-specific works (e.g. Tortonese, 1956, Italian waters; Capapé, 1977, Toulon waters; Barrull et. al., 1999, from Catalan littoral; Cugini & De Maddalena, 2003, off Pescara, Italy; Lipej et al., 2004, Adriatic Sea; Serena, 2005, the entire Mediterranean basin).

The first records of *A. vulpinus* from Turkish waters date back to the early 20<sup>th</sup> century (Ninni, 1923, Devedjian, 1926, as *Alopias vulpes* in both references). Thresher shark is quite common in Turkish Mediterranean, Aegean and Marmaric waters (Akşıray, 1987; Kabasakal, 2002, 2003; Kabasakal & Kabasakal, 2004), and occasional records have been reported from the prebosphoric waters in the Black Sea (Kabasakal, 1998).

In the present study, incidental captures of alopiid sharks by coastal nets in Turkish waters are reported, and the current status of conservation of alopiids is discussed.

# **MATERIAL AND METHODS**

The present study is part of an extensive research on sharks from Turkish waters, which has been carried out by Ichthyological Research Society (IRS) since 2000. Data on thresher sharks have been collected from the following sources: (a) scientific literature; (b) daily newspapers, fishing magazines and other popular me-

dia, and, as far as popular sources are concerned, the validity of the recordings has been confirmed by means of direct contact with the fishermen reported in the source; (c) visiting the fishing ports. For each examined thresher shark, the following data were recorded: total length (TL), weight (W), sex, date and locality, fishing gear, depth and time of the day. Photographs of the specimens, teeth samples (sp. no. 3, Tab. 1), set of upper and lower jaws of *Alopias superciliosus* (sp. no. 2, Tab. 1) and other relevant pieces of evidence about the catch are kept in the archives of IRS.

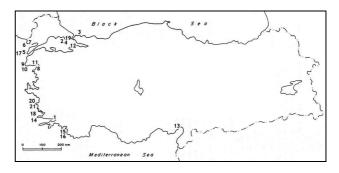


Fig. 1: Capture localities of alopiid sharks along Turkish coast. Numbers are same as in Table 1.

Sl. 1: Lokalitete vzdolž turške obale, na katerih so bile ujete obravnavane morske lisice. Številke so iste kot v Tabeli 1.

## **RESULTS**

Thresher sharks captured in Turkish waters are listed in Table 1, with fishing localities shown in figure 1. One of the two specimens of Alopias superciliosus was captured in the south-eastern Aegean Sea, the other off the northern coast of the Sea of Marmara (Fig. 1). Eleven specimens of Alopias vulpinus were captured along Aegean coast of Turkey, 3 specimens in Marmaric waters and Mediterranean, and 1 in prebosphoric waters in the Western Black Sea (Fig. 1). Among 19 thresher sharks, 7 specimens were captured, in order of importance, by trammel-netters, 7 other thresher sharks by gill-netters, 3 of them by purse-seiners, and the remaining 2 by driftnetters for swordfish (Tab. 1). Stationary nets were the most important fishing gear, as far as incidental captures of A. vulpinus in Turkish coastal waters are concerned. Gökova Bay specimen of A. superciliosus was entangled in a shrimp net, a kind of dredging gear over the bottom, while Silivri specimen of the bigeye thresher shark was captured by means of a purse-seiner, operated only a few kilometres off the coast line. Five (23.8%) alopiid sharks (specimens 1, 4, 6, 20 & 21 in Tab. 1) were captured in early morning before dawn.

Tab. 1: Alopiid sharks recorded in the present study. Specimen numbers are same as on the map in figure 1. Tab. 1: Morske lisice, opisane v pričujoči študiji. Številke primerkov so iste kot na zemljevidu na sliki 1.

| No                                 | TL (cm)                             | W (kg) | Sex      | Date       | Locality     | Fishing gear | Remarks   |  |  |  |  |
|------------------------------------|-------------------------------------|--------|----------|------------|--------------|--------------|---|--|--|--|--|
| Alopias superciliosus (Lowe, 1839) |                                     |        |          |            |              |              |   |  |  |  |  |
| 1                                  | 350                                 | 150    | ?        | 23.5.2005  | Gökova       | Shrimp-net   | Captured in coastal waters at 12 m depth,   |  |  |  |  |
|                                    |                                     |        | •        |            |              |              | early in the morning.   |  |  |  |  |
| 2                                  | 450                                 | ?      | ?        | 23.2.2007  | Silivri      | Purse-seine  | Captured a few kilometers off the coast line (Fig. 2)   |  |  |  |  |
|                                    | Alopias vulpinus (Bonnaterre, 1788) |        |          |            |              |              |   |  |  |  |  |
| 3                                  | 453                                 | ?      | 9        | 8.11.1996  | Şile         | Purse-seine  | The shark was captured in a purse-seine full of blue fish (Pomatomus saltator). Recorded by Kabasakal (1998). |  |  |  |  |
| 4                                  | 190                                 | ?      | 8        | 12.4.1997  | Silivri      | Gill-net     | According to fisherman, it was captured early in the morning before dawn. Recorded by Kabasakal (2003).       |  |  |  |  |
| 5                                  | 400                                 | 150    | ?        | 17.2.1999  | Çanakkale    | Gill-net     | Captured in coastal waters.   |  |  |  |  |
| 6                                  | 450                                 | ?      | <b>%</b> | 18.4.2001  | Enez         | Gill-net     | Captured in coastal waters, only 100 meters from the shoreline, early in the morning.                         |  |  |  |  |
| 7                                  | 450                                 | 120    | 2        | 04.03.2003 | Bay of Saroz | Trammel-net  | Captured in coastal waters.   |  |  |  |  |
| 8                                  | 600                                 | >500   | ?        | 25.12.2003 | Akçay        | Gill-net     | Captured in coastal waters.   |  |  |  |  |
| 9                                  | 400                                 | 120    | 9        | 20.01.2004 | Ezine        | Gill-net     | Captured in coastal waters.   |  |  |  |  |
| 10                                 | 250                                 | ?      | 8        | 03.04.2004 | Babakale     | Gill-net     | Captured in coastal waters.   |  |  |  |  |
| 11                                 | 500                                 | 300    | 9        | 15.05.2004 | Edremit      | Gill-net     | Captured in coastal waters.   |  |  |  |  |
| 12                                 | 350                                 | 200    | ?        | 01.10.2004 | Yalova       | Purse-seine  | Captured in near shore waters between Prince Islands and Anatolian coast.                                     |  |  |  |  |
| 13                                 | 500                                 | 320    | 0+       | 11.03.2006 | Karataş      | Trammel-net  | Captured in coastal waters.   |  |  |  |  |
| 14                                 | 300                                 | 100    | 9        | 27.03.2006 | Bodrum       | Trammel-net  | Captured in coastal waters.   |  |  |  |  |
| 15                                 | 600                                 | 500    | 0+       | 01.07.2006 | Fethiye      | Drift-net    | Captured in near shore waters, in a drift-net for swordfish.  |  |  |  |  |
| 16                                 | 500                                 | 400    | ?        | 09.07.2006 | Fethiye      | Drift-net    | Captured in near shore waters, in a drift-net for swordfish.  |  |  |  |  |
| 17                                 | 320                                 | 320    | ₹0       | 03.11.2006 | Bozcaada     | Trammel-net  | Captured between the eastern coast of the island and Gallipoli Peninsula.                                     |  |  |  |  |
| 18                                 | 400                                 | 120    | 9        | 26.12.2006 | Bodrum       | Trammel-net  | Captured in coastal waters.   |  |  |  |  |
| 19                                 | 300                                 | 85     | 9        | 20.01.2007 | Silivri      | Trammel-net  | Captured in coastal waters (Fig. 3).  |  |  |  |  |
| 20                                 | 400                                 | ?      | ?        | 05.02.2007 | Didim        | Gill-net     | Captured in coastal waters, early in the morning before dawn.   |  |  |  |  |
| 21                                 | 400                                 | 500    | 4        | 13.02.2007 | Didim        | Gill-net     | Captured in coastal waters, early in the morning around 4 o'clock.  |  |  |  |  |

# **DISCUSSION AND CONCLUSIONS**

The specimen of *Alopias superciliosus*, captured in Gökova Bay on 23 May 2005, was the first record of the bigeye thresher shark in Turkish waters. The second specimen of this species (Fig. 2) was captured on 23 February 2007, off the coast of Silivri, northern Sea of Marmara (Kabasakal & Karhan, *in press*). According to Compagno (1984), *A. superciliosus* is found in coastal waters over the continental shelves, sometimes close inshore in shallow waters. Nakano *et al.* (2003) reported that the bigeye thresher shark is a deep-water species, found in both coastal waters and the high seas, from the

surface to near the bottom at depths greater than 500 m. In the eastern Pacific Ocean, the observed maximum recorded depth of 723 m is the deepest ever recorded for *A. superciliosus* (Nakano *et al.*, 2003). Gökova Bay specimen of bigeye thresher shark has been captured only a few hundred meters off the shoreline and at a depth of about 12 m. Identification of the Gökova Bay specimen is based on the photographs and as genital organs were not visible on the studied pictures, the author was not able to determine its' sex. On the other hand, the Silivri specimen was eviscerated and cut in parts, and identification was based on the morphology of the head. The genital organs of the second bigeye thresher



Fig. 2: Head of Alopias superciliosus (Lowe, 1839), captured off Silivri coast (sp. no. 2).

Sl. 2: Glava vrste Alopias superciliosus (Lowe, 1839), ujete v bližini Silivrija v Turčiji (vrsta št. 2).

shark were not visible either. However, regarding the total lengths of both bigeye thresher sharks, 350 cm and 450 cm TL, respectively, and the size of the adult males and females (270 to 400 cm TL for males, and 355 to 430 cm TL for females) reported by Compagno (1984), it seems that the Gökova Bay specimen was probably an adult male or a subadult female, whereas the Silivri specimen was an adult male or female. The data given by Compagno (1984) is global rather than regional, and because of this reason, a specific investigation is needed for a better understanding of the reproductive characteristics of *A. superciliosus* in Levantine basin waters.

Alopias vulpinus (Fig. 3) is one of the most common incidentally captured sharks in sword-fish fishery, operated in Turkish waters (Kabasakal, 1998). But the paucity of research on the biology of *A. vulpinus* in Turkish waters is clear. For the moment, there is no regulation set for the conservation of thresher sharks in the current fishery act. Thresher shark is considered an open water predator in most of the ichthyological works of Turkish waters; however, one of the remarkable contributions of the present study is the finding that *A. vulpinus* occurs in

Turkish coastal waters and may be incidentally captured by the coastal artisanal fishing gear as well.

Many authors reported on the occurrence of A. vulpinus coastal insular or shelf waters (Compagno, 1984; Quignard & Capapé, 1971; Hattour & Nakamura, 2004; Lipej et al., 2004). Young thresher sharks often close inshore and in shallow bays (Compagno, 1984; Lipej et al., 2004). Hattour & Nakamura (2004) reported on the occurrence of six juveniles of A. vulpinus, ranging 136 to 143 cm TL, which were incidentally captured by trammel-netters from the Gulf of Tunis. Regarding the sizes of adult males and females (319 to 420 cm TL, for males, and 376 to 549 cm TL for females) reported by Compagno (1984), specimens 4 and 10 (Tab. 1) were juveniles, and their coastal occurrence coincided with the data reported by Compagno (1984). Based on the above data, the two males (sp nos. 6 and 17, Tab. 1) were adults. Ten thresher sharks (53.1%, Tab. 1) were females, and again based on the above reproductive data of Compagno (1984) and recorded total lengths in



Fig. 3: Thresher shark, Alopias vulpinus (Bonnaterre, 1788), captured off Silivri coast, on display at a fish store in Istanbul (sp. no. 19).

Sl. 3: Morska lisica Alopias vulpinus (Bonnaterre, 1788), ujeta v bližini Silivrija in razstavljena v neki istanbulski ribarnici (vrsta št. 19).

Hakan KABASAKAL: INCIDENTAL CAPTURES OF THRESHER SHARKS (LAMNIFORMES: ALOPIIDAE) FROM TURKISH COASTAL WATERS, 23–28

the present study, 8 of the 9 females (specimens 3, 7, 9, 11, 13, 15, 18 & 21) appeared to be adults. For the moment, the current data is not yet clear, whether those females penetrated coastal waters for reproduction or feeding; however, the fishing pressure caused by coastal fishing gear on the adult stock of *A. vulpinus* is obvious. A similar situation regarding the fishing pressure caused by the small-scale fishery on thresher sharks has been reported from Tunisian waters. According to Hattour & Nakamura (2004), the abundance of thresher shark in Tunisian waters apparently decreased dangerously. Authors reported that small-scale fishery seems targeting currently the vulnerable neonate of *A. vulpinus*.

Regarding the seasonality of captures, it was observed that thresher sharks have been captured in coastal waters almost throughout the year (Tab. 1). In order to regulate the coastal fishing activities and to provide rules of management for the conservation of thresher sharks, it is necessary to understand the nature and seasonality of this coastal occurrence.

According to Vas (1995), the success of sharks as a group, in evolutionary terms, is directly attributable to their adopted life history. In ecological terms, sharks are *k*-selected species, which means that they are maturing in very late ages and the litter size is small, a critical point making sharks vulnerable to overfishing.

According to FAO classification for the conservation and exploitation of fish resources, the conservation status of A. superciliosus and A. vulpinus is defined as, B3 (vulnerable to overfishing) and B4 (locally declined or extinct), respectively (Serena, 2005). Moreover, in the Mediterranean Sea, A. vulpinus is listed in the IUCN/ SSG Red List (Soldo, 2003). Thresher sharks are known to be incidentally captured by long-lining and driftnetting for tuna, swordfish and other pelagic bony fishes (Soldo, 2003; Serena, 2005). Pressure of coastal fishery on large sharks is generally neglected; however, incidental captures of large sharks by coastal nets have been recorded from different regions (Brewster-Geisz & Miller, 2000; Lipej et al., 2000; Kabasakal, 2004). Therefore, the effects of coastal fishery on large sharks should be estimated carefully, to achieve a clearer picture of incidental captures of sharks, as a whole. Although thresher sharks are regularly captured by both pelagic and coastal fishermen, there have been no attempts made in order to set regulatory measures for their conservation. This was because of the paucity of biological data on thresher sharks, as well as the passion of commercial fishermen to capture and land large sharks for display. Therefore, it is necessary to encourage fishermen to release incidentally captured thresher sharks, and other large sharks as well.

# MORSKE LISICE (LAMNIFORMES: ALOPIIDAE), NAKLJUČNO UJETE V TURŠKIH OBREŽNIH VODAH

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# **POVZETEK**

Po naključju je bilo v obrežnih vodah Turčije ujetih 21 morskih lisic (Alopiidae): 2 osebka vrste Alopias superciliosus in 19 osebkov vrste Alopias vulpinus. Avtor podaja splošne informacije o ujetih lisicah in razpravlja o njihovem trenutnem ohranitvenem statusu.

Ključne besede: Alopiidae, Alopias superciliosus, Alopias vulpinus, omejeno ribištvo, varstvo, turške obrežne vode

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# SIZES OF EIGHT OVIPAROUS ELASMOBRANCH SPECIES HATCHED IN TWO MEDITERRANEAN AREAS: A SURVEY AND RECENT DATA

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# **ABSTRACT**

In the present paper, the authors report on the size of eight oviparous elasmobranch species hatched in two Mediterranean areas: off the Tunisian coast (central Mediterranean) and Languedocian coast (southern France, northern Mediterranean). Most of the observations were made in experimental conditions, from egg cases placed in tanks. For each species, no intraspecific variation was observed between specimens from both areas. In contrast, new hatched smallspotted catsharks from the Mediterranean appeared to be smaller than those from the Atlantic.

Key words: Elasmobranchs, size at hatching, oviparous species, Tunisian coast, Languedocian coast, Mediterranean

# GRANDEZZA DI OTTO SPECIE OVIPARE DI ELASMOBRANCHI, SCHIUSE IN DUE AREE MEDITERRANEE: REVISIONE E DATI RECENTI

# SINTESI

Gli autori riportano la grandezza di otto specie ovipare di elasmobranchi, schiuse in due aree mediterranee: in prossimità della costa tunisina (Mediterraneo centrale) e della costa di Languedoc (Francia meridionale, Mediterraneo settentrionale). La maggioranza delle osservazioni è stata fatta su uova tenute in vasca, in condizioni sperimentali. Non è stata registrata alcuna variazione intraspecifica fra gli individui della prima e della seconda area di studio, per nessuna specie. Per il gattuccio viene segnalato che i giovani esemplari provenienti dal Mediterraneo appaiono più piccoli di quelli atlantici.

**Parole chiave:** Elasmobranchi, grandezza alla schiusa, specie ovipare, costa tunisina, costa di Languedoc, Mediterraneo

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# **INTRODUCTION**

In oviparous elasmobranch species, size at hatching (rather than size at birth) could be recorded only from embryos at the end of development found in egg cases or in recent hatched specimens. Unfortunately, such findings in the wild have been rarely reported in literature. Information on the size at hatching in oviparous elasmobranch species has been mostly provided from experimental observations, carried out on embryonic development in egg cases placed in tanks, or laid by females in captivity. In this paper; we give observations on the size at hatching in 8 oviparous elasmobranch species collected off two Mediterranean areas: the Tunisian (central Mediterranean) and Languedocian coasts (southern France, northern Mediterranean).

# **MATERIAL AND METHODS**

Observations were conducted between 1970 and 2005 on 8 oviparous elasmobranch species from the above mentioned Mediterranean areas (Fig. 1). Of the 8 oviparous elasmobranch species presented in this paper, 5 species occur in both areas: 3 scyliorhinids, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810, the smallspotted catshark *Scyliorhinus canicula* (Linnaeus, 1758), the nursehound *S. stellaris* (Linnaeus, 1758) and 3 rajids, the starry ray *Raja asterias* Delaroche, 1809, the thornback ray *R. clavata* Linnaeus, 1758 and the speckled ray *R. polystigma* Regan, 1923; 2 other rajids occur only off the Tunisian coast: the brown ray *R. miraletus* Linnaeus, 1758, and the rough ray *R. radula* Delaroche, 1809.

Observations and measurements were made on developing embryos removed from egg cases found in the wild, specimens hatched from egg cases deposited in tanks or laid by captive females, smallest free-swimming specimens exhibiting or not remains of internal yolk. Measurements included total length (TL) for scyliorhinids following Compagno (1984) and disc width (DW) for rajids following Clark (1922, 1926) recorded to the nearest millimetre and mass recorded to the nearest decigram.

Wild specimens were collected from trawling performed from off both Tunisian and Languedocian coasts. Egg cases were deposited in tanks located at three sites. The first site was the Institut National des Sciences et Technologies de la Mer of Salammbô (INSTM), the city 15 km north of Tunis (Tunisia); the second site was the aquarium of La Grande Motte, the city located 25 km southeast from Montpellier (France), whereas the third site was the Laboratoire d'Ichtyologie of the Université Montpellier 2 in Montpellier.

Tanks of the first and second sites contained 60 litres and were supplied with water directly drawn from the sea; the flow was 150 litres per hour. The tank of the third site contained 120 litres; it was regularly supplied by marine water collected from the sea and immediately carried to the laboratory.

For each species, we cite vernacular name in English (En), French (Fr) and Arabic Tunisian language (Tn); we also give sizes at first sexual maturity, maximal size and size of the smallest free-swimming specimens when available, size (length x width) and weight of egg cases, previous and recent observations carried out on hatched specimens, or in some cases in embryos at the end of embryonic development.

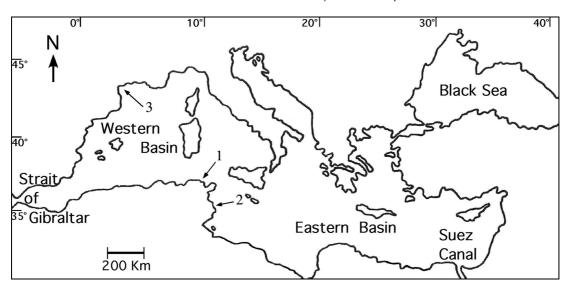


Fig. 1: Map of the Mediterranean Sea showing the areas were female oviparous elasmobranchs were collected. Arrows 1 and 2: Tunisian coast; arrow 3: Languedocian coast.

Sl. 1: Zemljevid Sredozemskega morja z območjema, v katerih so bile ujete samice jajcerodnih morskih psov in skatov. Puščici 1 in 2: tunizijska obala; puščica 3: languedoška obala.

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# **RESULTS**

# **Family Scyliorhinidae**

# Galeus melastomus Rafinesque, 1810

blackmouth catshark (En), chien espagnol (Fr), gattous bhar (Tn)

Off the Tunisian coast, the blackmouth catshark is abundantly caught off southern areas, at depth between 200 and 600m, its southernmost extension being the Gulf of Hammamet (Capapé & Zaouali, 1977; Bradaï et al., 2000). Males were sexually mature at the size above 400 mm TL, females between 390 and 420 mm TL, whereas the largest male and the largest female were 560 and 550 mm TL, respectively (Capapé & Zaouali, 1977). Off the Languedocian coast, the species is captured at lower depth than the Tunisian specimens, i.e. between 120 and 200 m. Males and females sexually matured between 510–550 mm and females between 520–610 mm total length (TL), respectively, while the largest male and the largest female were 620 mm and 650 mm TL, respectively (unpubl. data).

Egg cases from the Tunisian coast were 42-48 mm in length, 18-25 mm in width and weighed 3.7-4.4 g (Capapé & Zaouali, 1977), while egg cases from the Languedocian coast were 36-50 mm in length, 14-21 mm in width and weighing 3.5-5.0 g. Egg cases removed from females of both areas were placed in tanks, but they rapidly degenerated after a week maximum. Egg cases caught off the second area contained developing embryos, which quickly died. They were sorted from the cases, were probably term embryos, both measuring 75 mm TL and weighing 2.9 and 3.2 g. The smallest free-swimming specimens found off the Languedocian coast were 2 males, 125 and 145 mm TL, both weighing 8 g, and two females, 135 and 140 mm TL, weighing 6 g and 8 g, respectively. The smallest blackmouth catshark recorded to date was 9 mm TL, off the south coast of Portugal (Costa et al., 2005).

# Scyliorhinus canicula (Linnaeus, 1758)

smallspotted catshark (En), petite roussette (Fr), gattous bhar (Tn)

The smallspotted catshark is probably the elasmobranch species most commonly landed in both areas (Capapé, 1977a; Capapé et al., 2000). Off the Tunisian coast, males were sexually mature at 400 mm TL, females between 400 and 450 mm TL, while the largest male and the largest female were 580 and 560 mm TL, respectively (Capapé, 1977a). Off the Languedocian coast, males and females sexually matured between 430–440 mm and 410–450 mm total length (TL), respectively, while the largest male and the largest female were 550 mm and 510 mm TL, respectively (unpubl. data).

Egg cases from the Tunisian coast were 38-48 mm in length, 14-19 mm in width and weighing 2.3-4.2 g (Capapé, 1977a), while egg cases from the Languedocian coast were 41-58 mm in length, 16-20 mm in width and weighing 4.1-5.9 g. Capapé (1977a) provided information concerning size at hatching from egg cases placed in tanks of the INSTM, and noted that the incubation period decreased with temperature, 271-285 days with a temperature range between 14-19.5 °C, and 177-180 days with temperature range between 19-24 °C. However, no difference was observed between the 5 hatched specimens, a single male and 4 females ranged 84-88 mm TL and weighed 2.1-2.2 g. Of the 8 egg cases removed from females and placed in tanks in the Laboratoire d'Ichtyologie of Université Montpellier 2 with temperature range between 19-21 °C, a single egg hatched after the incubation period of 145 days; the female recorded was 78 mm TL and weighing 1.9 g. Of 3 egg cases with developing embryos placed in the same tank, one aborted, while from the other two a male and a female were hatched two weeks later, measuring 80 mm and 78 mm in length, and weighing 2.0 and 1.8 g, respectively. Ellis & Schackley (1997) noted that eggs took 5-6 months to hatch at water temperature range of 8.5-18.1 °C. Neonates ranged 90-112 mm and weighed 2.6-3.9 g.

# Scyliorhinus stellaris (Linnaeus, 1758)

nursehound (En), grande roussette (Fr), gattous bhar el kbir (Tn)

The nursehound is captured off the Tunisian coast less often than the smallspotted catshark (Capapé, 1977b). Moreover, it is considered rare in southern Tunisian areas such as the Gulf of Gabès (Bradaï *et al.*, 2002). Off the Tunisian coast, males and females were sexually mature at 770 mm TL and 790 mm TL, respectively, while the largest male and the largest female were 1080 and 1115 mm TL, respectively (Capapé, 1977b). Off the Languedocian coast, Capapé *et al.* (2000) recorded some specimens and suggested that the species was not so abundant in the area as in the past (Moreau, 1881; Euzet, 1959). Of the 6 specimens observed, an adult female 980 mm TL bore two egg cases, one per oviduct (Capapé *et al.*, 2000).

Egg cases from the Tunisian coast were 92–96 mm in length, 37–39 mm in width and weighing 28.2–29.5 g (Capapé, 1977b), while egg cases from the Languedocian coast were 94 mm in length, 37 mm in width and weighing 29 g (Capapé et al., 2000). Of the 10 egg cases placed in tanks from INSTM of Salammbô, only two were hatched after an incubation period of 198 and 201 days. One male and one female measured 108 mm TL and 107 mm TL, respectively, both weighing 4.5 g. Size at hatching for specimens from the Languedocian coast were from 105 to 110 mm TL according to Capapé et al.

(2006a), who noted that the incubation period ranged between ten and twelve months, in agreement with Moreau (1881) and Ehrenbaum (1927) for *S. stellaris* from the Atlantic and North Sea. Moreover, neonates from the Adriatic Sea were larger, as the size at hatching was between 130 and 163 mm according to Skaramuca & Prtenjaca (1985). These differences may be due to fact that embryos developed in egg capsules deposited in tanks at Salammbô and La Grande Motte, and in natural environment in the Adriatic Sea.

# **Family Rajidae**

# Raja asterias Delaroche, 1809

starry ray (En), raie étoilée (Fr), kerchella (Tn)

The starry ray is commonly captured off the northern coast of Tunisia from the Algerian border to the Gulf of Tunis and it is unknown southward (Capapé, 1977c). Males and females were sexually mature when over 360 mm and 430 mm DW, respectively, while the largest male and the largest female were 470 and 520 mm DW, respectively (Capapé, 1977c). Off the Languedocian coast, preliminary observations reported by Capapé *et al.* (2006b) showed that males and females were adult when over 330 mm and 360 mm DW, respectively.

Egg cases from the Tunisian coast were 103–110 mm in length with horns, 45–48 mm in length without horns, 34–37 mm in width and weighing 9.4 g (Capapé, 1977c), while egg cases from the Languedocian coast were: length with horns between 9.6 and 105 mm, length without horns between 43 and 47 mm, width between 32 and 34 mm, and weighing between 9.2 and 9.7 g (Capapé *et al.*, 2006b). Of 10 egg cases placed in tanks at the INSTM, only two were hatched after 154 and 147 days, a female 67 mm DW, 6.5 g, and a male 68 mm DW, 6.6 g, respectively. Egg cases of Languedocian *R. asterias* were not placed in tanks, however, a juvenile male, 72 mm DW, 7.9 g, with remains of internal yolk was recorded, suggesting a recently hatched specimen and the smallest starry ray recorded to date in the area.

# Raja clavata Linnaeus, 1758

thornback ray (En), raie boűclée (Fr), kerchella (Tn)

The thornback ray is commonly captured throughout the coast of Tunisia from the Algerian to the Libyan borders (Capapé, 1976; Bradaï et al., 2004). Males and females were sexually mature when over 480 mm and 540 mm DW, respectively, while the largest male and the largest female were 640 and 680 mm DW, respectively (Capapé, 1976). Off the Languedocian coast, preliminary observations reported by Capapé et al. (2006b) showed that males and females were adult when over 330 mm and 360 mm disc width, respectively. R. cla-

vata was reported off the Languedocian coast by people from Doumet (1860) to Quignard (1965), where it was previously very common. In contrast, recent investigations conducted by Capapé et al. (2007a) in the area showed that captures remain rather occasional. The smallest male and female adults were 420 mm and 540 mm DW, respectively, whereas the largest male and largest female were 510 mm and 690 mm DW. Production of egg cases was observed throughout the year, except in April and August.

Egg cases from the Tunisian coast were 121–135 mm in length with horns, 70–78 mm in length without horns, 50–54 mm in width and weighing 13–18 g (Capapé, 1976), while egg cases from the Languedocian coast were between 122–127 mm long with horns, 61–66 mm long without horns, 50 and 56 mm wide, and weighing between 19.5 and 22.5 g.

Of 10 egg cases placed in tanks from the INSTM, only two were hatched after 148 and 142 days, two females 75-76 mm DW and 7.9-8.0 g (Capapé, 1976). No egg case from the Languedocian coast was placed in tanks; the smallest free-swimming specimens recorded to date in the area, a male and a female, were both 110 mm DW and weighed 31 g. Clark (1922) noted that the incubation period in laboratory tanks was between 4 and 5.5 months; 23 embryos were hatched, with DW ranging from 65 to 85 mm (these data were in agreement with those recorded by Capapé (1976)). Further, Ellis & Schackley (1995) carried out studies in aquarium on R. clavata from the Bristol Channel. They noted that the incubation period lasted less than 7 weeks, and that total mean length, disc width and mass of newly hatched specimens were 118, 75 mm and 8.9 g, respectively.

# Raja miraletus Linnaeus, 1758

brown ray (En), raie miroir (Fr), kerchella (Tn)

The brown ray is commonly captured throughout the coast of Tunisia from the Algerian to the Libyan borders (Capapé & Quignard, 1974; Bradaï et al., 2004). Males and females from the Gulf of Tunis were sexually mature when over 220 mm and 240 mm DW, respectively, while the largest male and the largest female were 320 mm and 330 mm DW, respectively (Capapé & Quignard, 1974). R. miraletus was reported off the Languedocian coast by researchers from Doumet (1860) to Quignard (1965). Euzet (1959) and Quignard (1965) recorded some specimens only and considered the occurrence of the species very rare in the area. Capapé et al. (2006b) noted that no specimen was recorded in the area since Quignard (1965). In contrast, Capapé et al. (2007b) noted that R. miraletus is commonly landed at fishing sites along the Senegalese coast. Adult males and females are mostly captured in spring and summer. The smallest sexually mature male and female were 270 mm

and 310 mm DW, respectively. The largest male and the largest female adults were 380 mm and 415 mm DW, respectively. They were the largest *R. miraletus* reported to date for both males and females.

Egg cases of Tunisian brown rays were 84–88 mm in length with horns, 42–47 mm in length without horns, 27–32 mm in width, and weighing 4.9–6.0 g. Egg cases of Senegalese brown rays were 88–97 mm in length with horns, 48–52 mm without horns, their widths were 28–32 mm, and they weighed 8.7–9.9 g. Several eggs were placed in tanks from the INSTM of Salammbô, and 14 were hatched. Temperature varied between 14 and 24.5 °C. The incubation period lasted for 123–135 days. Seven females and 7 males were hatched, measuring between 60 and 64 mm DW and weighing 3.9–4.2 g (Capapé & Quignard, 1974).

#### Raja polystigma Regan, 1923

speckled ray (En), raie tachetée (Fr), kerchella (Tn)

The speckled ray is abundantly caught off the northern coast of Tunisia from the Algerian border to the Gulf of Tunis, southward the species is occasionally captured (Capapé & Quignard, 1978). Males and females were sexually mature when over 340 mm and 400 mm DW, respectively, while the largest male and the largest female were 450 and 470 mm DW, respectively (Capapé & Quignard, 1978). Off the Languedocian coast, *R. polystigma* was only reported by Quignard (1965), however, no specimen was available for confirmation. The specimen described by Capapé *et al.* (2006c) confirmed the occurrence of the species in the area.

Egg cases of Tunisian *R. polystigma* were 103–110 mm in length with horns, 45–48 mm in length without horns, 34–37 mm in width, and weighing 9.4g (Capapé & Quignard, 1978). Twelve egg cases were placed in tanks of the INSTM of Salammbô, and they were all hatched. Moreover, the smallest Tunisian specimen observed was 110 mm in disc width and weighing 22 g.

# Raja radula Delaroche, 1809

rough ray (En), raie râpe (Fr), kerchella (Tn)

The rough ray is commonly caught off the Tunisian coast from the Algerian to the Libyan borders and entered brackish waters (Capapé, 1974; Capapé *et al.*, 2004; Mejri *et al.*, 2004). Males and females were sexually mature when over 320 mm and 340 mm DW, respectively, while the largest male and the largest female were 400 and 420 mm DW, respectively (Capapé, 1974). *Raja radula* has never been recorded off the Languedocian coast.

Egg cases of Tunisian R. radula were 100-120 mm in

length with horns, 51–57 mm in length without horns, 34–37 mm in width, and weighing 9.6–10.5 g (Capapé, 1974). Several eggs were placed in tanks from the INSTM of Salammbô, and 11 were hatched. Temperature varied between 14 and 24 °C. The incubation period lasted for 134–148 days. Six females and 5 males were hatched, measuring between 61 and 66 mm DW and weighing 6.0–6.3 g (Capapé, 1974). The smallest free-swimming Tunisian rough rays were found in a brackish area, the Tunis Southern Lagoon, by Mejri *et al.* (2004). These were two females 120 mm and 135 mm DW, respectively, weighing 14 g and 15 g, respectively.

#### **DISCUSSION**

Observations conducted over a period of three decades in two Mediterranean areas show that information on the size at hatching of elasmobranch oviparous species was very rare and concerned only experimental observations. Observations from other Mediterranean areas and outside the Mediterranean were also very rare; interand intraspecific comparison remain difficult to assess.

Size at hatching is related to size of egg cases in elasmobranch oviparous species. A good instance was given by observation in the smallspotted catshark. Egg cases from the Atlantic specimens of Scyliorhinus canicula found in British waters were larger than those from the Mediterranean (off both Tunisian and Languedocian coasts); consequently, newly hatched specimens were larger in the first area than in the second, matured at a larger size and reached larger maximal size (Capapé et al., 1991; Ellis & Schackley, 1997) and are in agreement with Leloup & Olivereau's findings (1951). Similar patterns were observed in Galeus melastomus and Scyliorhinus stellaris. In contrast, egg cases, size at hatching, size at sexual maturity and maximal size were practically the same for rough rays from British waters (Clark, 1922, 1926; Steven, 1936; Holden et al. 1971, Holden, 1975) and for specimens from both Tunisian and Languedocian coasts (Capapé, 1976; Capapé et al., 2007a). However, Raja clavata from the Adriatic Sea were smaller (Jardas, 1973), due probably to the fact that temperature and salinity are higher in the latter area (Dulčić & Grbec, 2000). These intraspecific changes suggest that larger specimens probably give larger egg cases, which consequently provide larger specimens (see Mellinger et al., 1984).

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# VELIKOST OSMIH JAJCERODNIH VRST MORSKIH PSOV IN SKATOV, IZVALJENIH V DVEH SREDOZEMSKIH OBMOČJIH: PREGLED IN NOVEJŠI PODATKI

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#### **POVZETEK**

Avtorji članka poročajo o velikosti osmih jajcerodnih morskih psov in skatov, izvaljenih v dveh sredozemskih območjih: v bližini tunizijske (srednje Sredozemlje) in laguedoške obale (južna Francija, severno Sredozemlje). Večino opažanj so zabeležili v eksperimentalnih okoliščinah, in sicer na jajcih v rezervoarjih. Pri preučevanih vrstah ni bila opažena nobena intraspecifična variacija med primerki iz obeh območij. Po drugi strani pa so bile novorojene navadne morske mačke iz Sredozemlja videti manjše kot atlantske.

**Ključne besede:** Elasmobranchii, velikost ob izvalitvi, jajcerodne vrste, tunizijska obala, languedoška obala, Sredozemlje

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# PYRETHRUM (TANACETUM CINERARIIFOLIUM) FROM THE NORTHERN ADRIATIC AS A POTENTIAL SOURCE OF NATURAL INSECTICIDE

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#### **ABSTRACT**

Pyrethrum Tanacetum cinerariifolium (Trevir.) Schultz-Bip., a species native to the Eastern Adriatic coastal mountains and islands, is a plant widely used in the production of natural insecticides, pyrethrins. The biosynthetic potential of Pyrethrum from two different locations in the Northern Adriatic for pyrethrin production was determined. In all the samples obtained, all 6 pyrethrins were detected, as measured by HPLC. The highest pyrethrin content was detected in the flower heads, which contained on average 1.2% pyrethrins of dry weight. The pyrethrin content of flower heads from the Northern Adriatic populations is comparable with the content levels in conventional production of Pyrethrum, but not as high as the content levels for highly productive Pyrethrum clones from countries currently producing Pyrethrum.

Key words: Pyrethrum, Tanacetum cinerariifolium, pyrethrins, HPLC, Northern Adriatic

# PIRETRO (TANACETUM CINERARIIFOLIUM) DEL NORD ADRIATICO COME FONTE POTENZIALE DI INSETTICIDI NATURALI

# SINTESI

Il piretro, Tanacetum cinerariifolium (Trevir.) Schultz-Bip., è una specie nativa delle montagne a ridosso dell'Adriatico orientale e delle isole, ampiamente usato nella produzione di insetticidi naturali quali le piretrine. È stato pertanto determinato il potenziale biosintetico, per la produzione di piretrine, del piretro proveniente da due zone del Nord Adriatico. In tutti i campioni analizzati, con l'ausilio dell'HPLC, sono state trovate tutte e sei le piretrine. Il contenuto di piretrine maggiore è stato riscontrato nei capolini, che contengono in media 1,2% di piretrine (peso secco del campione). Il contenuto di piretrine dei capolini raccolti nelle zone del Nord Adriatico è paragonabile ai livelli ottenuti tramite produzione tradizionale di piretrine, ma non raggiunge i livelli ricavati dagli altamente produttivi cloni di piretro, utilizzati nei paesi che attualmente producono piretrine.

Parole chiave: piretro, Tanacetum cinerariifolium, piretrine, HPLC, Nord Adriatico

#### **INTRODUCTION**

Pyrethrum *Tanacetum cinerariifolium* (Trevir.) Schultz-Bip., previously classified within the genus *Chrysanthemum*, and with a still commonly used (Obukosia *et al.*, 2005) synonym *Chrysanthemum cinerariaefolium* Vis., Asteraceae (Figs. 1a, b), is a plant widely used for natural insecticide production (Hitmi *et al.*, 2000). It is the only agronomically important source of pyrethrins, identified also in other members of the genus *Tanacetum* and other genera of the same family, such as *Calendula*, *Chysanthemum*, *Tagetes* and others (Hitmi *et al.* 2000).

The term "pyrethrum" refers to the plant, flower head or flower extract, with the active insecticidal components of pyrethrum, being known as "pyrethrins" (Morris et al., 2006). Pyrethrins are a combination of six monoterpene esters (Keskitalo, 1999; Gspan et al., 2004). Pyrethrin I, cinerin I, and jasmolin I are collectively referred to as "pyrethrins I", whereas pyrethrin II, cinerin II, and jasmolin II are collectively referred to as "pyrethrins II". The typical extract contains pyrethrins, cinerins and jasmolins in the proportions 10:3:1 (Crombie, 1995), with the ratio of pyrethrins I to pyrethrins II (PyI/PyII) being typically around 1.0, although it can vary between 0.5 and 3.5 (Bhat, 1995). The activity of pyrethrins is a consequence of the esters mixture, depending on the ratio of Pyl/Pyll (Bhat, 1995). Pyrethrins affect the nervous system of insects, blocking nerve junctions and the action of voltagesensitive sodium channels (Sonderlund, 1995). The advantages of pyrethrins include effectiveness at low dosage, on a wide range of household and public health insects, rapid insecticidal action - knock-down and killing effects, repellency, less toxicity than other insecticides to mammals, and other homeotherms, rapid degradation on exposure to light and air, and the lack of bioaccumulation in food chains and ground water (Cohran, 1995; Jovetić & de Gooijer, 1995). To avoid instability in light and air, they are formulated with antioxidants, stabilizers and synergists (Jovetić & de Gooijer, 1995; Hitmi et al.

The Pyrethrum plant is a long-stemmed perennial plant, 45 cm to 60 cm in height, that blooms from spring to summer and that can be harvested for up to five years. The Pyrethrum flower head (Figs. 1b, c, d), the main source of pyrethrins, is a compound inflorescence consisting of two floret types: disc florets with yellow corollas in the centre of the head and ray florets, with white corollas from the head's outer rim (Bhat, 1995). Pyrethrins mostly accumulate in achenes (94%) located under the flower's receptaculum (Brewer, 1973) and in minor quantities in the disc florets, ray florets and in the receptacles (Fig. 1d) (Head, 1966). They accumulate in two types of secretory tissue in the system of secretory channels inside the achene wall (Fig. 1e) and in cells of the oil glands on the achene surfaces (Figs. 1e, f) and the leaves (Pal & Dhar, 1985). The flower heads are first cut and allowed to dry in the field. Pyrethrins from the dried flower heads are extracted with petroleum-based solvents in order to produce a dark oleoresin, which is then refined into a coloured extract without wax. Pyrethrin content depends on several factors: genotype, flower maturity, harvesting interval, climate, drying method (Zieg *et al.*, 1983) and storage conditions (Morris *et al.*, 2006). The highest reported pyrethrins content in selected high producing clones from Australia was 2.4% (Morris *et al.*, 2006), in Kenya 2.0% (Ikahu *et al.*, 1994) and India 2.5% (Ravishankar *et al.*, 1989; Rajasekaran *et al.*, 1993) of flower dry weight. It was also reported that selected Pyrethrum varieties contain up to 3.0% pyrethrins of flower dry weight (Hitmi *et al.*, 2000).

Pyrethrum is a species native to the area of the Eastern Adriatic coastal mountains and islands of the former SFR Yugoslavia (i.e. Slovenia, Croatia, Bosnia and Herzegovina, Montenegro) and Albania, although it has been cultivated and locally naturalized in other parts of Europe (Heywood, 2004). The cultivation of Pyrethrum for the production of insecticide pyrethrins started in the middle of the 19<sup>th</sup> century in the regions of the native species, which was part of the Austro-Hungarian Monarchy at that time. At the beginning of the 20<sup>th</sup> century, it was cultivated on more than 2,000 ha of coastal regions of the Eastern Adriatic, in Dalmatia and the islands (Kathe et al., 1993). These regions dominated in the cultivation of Pyrethrum for insecticide pyrethrins until World War I, when the plant was introduced to Japan. Later, other countries such as Kenya, Tanzania, India, Tasmania (Australia), and the USA became producers of pyrethrins. It is also cultivated in Europe, in Austria, Germany, France, Hungary, Italy, Spain and Russia (Keskitalo, 1999).

As an insecticide, pyrethrins are healthier and constitute an environmentally aware and efficient method of insect control (Jovetić & de Gooijer, 1995). Currently, pyrethrins are used mainly for protecting foodstuffs or for storage in the dark, for anti-lice shampoo and indoor sprays, and they are also approved for use on organic farms (Hitmi *et al.*, 2000). The world production of natural pyrethrins lags behind the demand from the global market (Hitmi *et al.*, 2000). Therefore data about the pyrethrin content of Pyrethrum from their native area could represent a basis for further studies of this agronomically important plant species.

The main purpose of our work was to determine the biosynthetic potential of native species from the area of the Adriatic coastal mountains and islands. The pyrethrins content of flower heads of Adriatic origin collected on the island of Cres, Croatia and in the Botanical Garden of Ljubljana, Slovenia, was determined. In addition, we determined the pyrethrins content in different plant parts, at different developmental stages of flowers, as well as in leaves, stems and roots.



Fig. 1: Pyrethrum Tanacetum cinerariifolium (Trevir.) Schultz Bip. from both locations. (a, b) flowering Pyrethrum plant; (c) capitulum with ¾-open disc florets reached maximum pyrethin content; (d) vertical cross section of flower head; (e) longitudinal cross section through disc florets ovary (200x); (f) disc florets with oil glands on the surfaces of the ovary wall.

Legend: o-df = open disc florets, c-df = closed disc florets, r = white rye florets, df = yellow disc florets, r = capitulum's flattened axis, sc = secretory channels, og = oil glands. Secretory channels and oil glands are the site of pyrethrins accumulation.

Sl. 1: Bolhač Tanacetum cinerariifolium (Trevir.) Schultz Bip. z obeh lokacij. (a, b) bolhač v polnem cvetju; (c) cvetne glavice dosegajo maksimalne vsebnosti piretrinov takrat, ko je odprtih ¾ cevastih cvetov; (d) vzdolžno prerezana cvetna glavica bolhača; (e) plodnica cevastega cveta prečno (200x); (f) cevasti cvetovi z oljnimi žlezami na steni plodnice.

Legenda: o-df = odprti cevasti cvetovi, c-df = zaprti cevasti cvetovi, rf = jezičasti cvetovi, df = cevasti cvetovi, r = cvetišče, sc = sekretorni kanali, og = oljne žleze na površini plodnice. Sekretorni kanali in oljne žleze so mesto akumulacije piretrinov.

#### **MATERIAL AND METHODS**

#### Plant material

Flower heads, leaves, stems and roots of Pyrethrum, *Tanacetum cinerariifolium* (Trevir.) Schultz-Bip. (Figs. 1a, b), of Adriatic origin, cultivated in the Botanical Garden of Ljubljana (Latitude 46°3′19N, Longitude 14°30′52E, Altitude 281 m), and years ago cultivated, wild grown on the island of Cres (Latitude 44°57′41N, Longitude 14°24′28E, Altitude 10 m), Croatia, were used for analysis. Pyrethrum flowers were collected in the middle of June in the years 1999 and 2000 in the Botanical Garden of Ljubljana and at the beginning of June 2000 on the island of Cres. Plant material, flowers and various plant parts were cold stored in the fridge (5 °C) for a few days or in the deep freeze (-80 °C) for a longer period until extraction procedures were performed.

# **Extraction and analysis of pyrethrins**

For the quantitative and qualitative determination of pyrethrins in various plant parts, high-pressure liquid chromatography (HPLC) was used (Gspan *et al.*, 2004). Briefly, pyrethrins were extracted with petroleum ether (40-60 °C) from plant material, crushed in a mortar with silica sand and anhydrous sodium sulphate, evaporated

to dryness, re-dissolved in  $CH_3CN$ , filtered through a 0.22 µm mesh filter and analysed using a Waters HPLC system with a diode array (PDA) detector. Separations were performed on a Nova Pack C18 column (Waters, 150 x 3.9 mm), using a gradient of solvent  $CH_3CN$  and Milli Q  $H_2O$  with a flow rate of 1.4 ml/min. Absorbance was monitored at 225 nm. Pyrethrins in the sample were identified on the basis of retention time and characteristic absorption spectra. The standards used (Figure 2a) were pyrethrins I (cinerin I, pyrethrin I, and jasmolin I), and pyrethrins II (cinerin II, pyrethrin II and jasmolin II) (pyrethrins technical mixture, PESTANAL, Riedel-de-Haën).

#### **Statistics**

The Student's t-test was used for evaluating levels of statistical significance (*P*) between samples of each pyrethrin from flowers of different origin.

# **RESULTS AND DISCUSSION**

# **Biosynthetic potential of native Pyrethrum**

The pyrethrins content in flower heads of native species from the area of the Adriatic coastal mountains and islands was studied (Fig. 1). In all flower samples

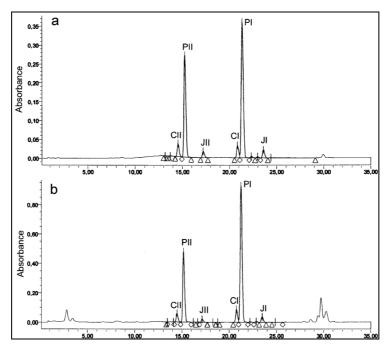


Fig. 2: (a) HPLC chromatogram of standard pyrethrin mixture; (b) HPLC chromatogram of pyrethrum flower head from the Ljubljana Botanical Garden.

Legend: CII = cynerin II, PII = pyrethrin II, JII = jasmolin II, CI = cynerin I, PI = pyrethrin I, JI = jasmolin I. SI. 2: (a) HPLC kromatogram standardne mešanice piretrinov; (b) HPLC kromatografska analiza piretrinov iz cvetnih glavic bolhača, nabranega v Botaničnem vrtu v Ljubljani.

Legenda: CII = cinerin II, PII = piretrin II, JII = jasmolin II, CI = cinerin I, PI = piretrin I, JI = jasmolin I.

Tab. 1: Pyrethrin content in various plant parts of Pyrethrum, collected in the Botanical Garden of Ljubljana and on the island of Cres: flower heads, leaves, stems, roots, in different parts of flower heads, open florets, closed florets and the receptaculums with the remaining parts of flower heads. Average (n = 3-5) contents of pyrethrins and standard deviations (SD) are shown. DW = dry weight.

Tab. 1: Vsebnost piretrinov v posameznih delih bolhača, nabranega v Botaničnem vrtu v Ljubljani in na otoku Cresu: cvetnih glavicah, listih, steblu, koreninah, v posameznih delih cvetne glavice, odprtih in zaprtih cvetovih ter razširjeni osi socvetja s preostanki cvetne glavice. Prikazana je povprečna vsebnost posameznih piretrinov (n = 3–5) in standardna deviacija (SD). DW = suha masa.

|               |               |                 |                |              | Pyrethrins     | (mg/g dry we  | eight) |                 |               |            |                      |
|---------------|---------------|-----------------|----------------|--------------|----------------|---------------|--------|-----------------|---------------|------------|----------------------|
| Sample        | Cinerin<br>II | Pyrethrin<br>II | Jasmolin<br>II | Cinerin<br>I | Pyrethrin<br>I | Jasmolin<br>I |        | Pyrethrins<br>I | PyI/<br>Py II | Pyrethrins | Pyrethrins<br>(% DW) |
| Flower heads* |               |                 |                |              |                |               |        |                 |               |            |                      |
| (botanical    |               |                 |                |              |                |               |        |                 |               |            | 1.2                  |
| garden)       | 0.97          | 6.65            | 0.19           | 0.42         | 4.06           | 0.12          | 7.81   | 4.59            | 0.59          | 12.40      | 1.2                  |
| SD            | 0.16          | 0.99            | 0.03           | 0.05         | 0.77           | 0.03          | 1.16   | 0.84            |               | 0.63       |                      |
| Flower heads* |               |                 |                |              |                |               |        |                 |               |            |                      |
| (Cres)        | 0.47          | 4.01            | 0.15           | 0.49         | 6.09           | 0.20          | 4.62   | 6.79            | 1.45          | 11.41      | 1.1                  |
| SD            | 0.04          | 0.27            | 0.02           | 0.03         | 0.50           | 0.01          | 0.32   | 0.54            |               | 0.63       |                      |
| Open disc     |               |                 |                |              |                |               |        |                 |               |            |                      |
| and ray       |               |                 |                |              |                |               |        |                 |               |            | 1 7                  |
| florets**     | 0.67          | 5.62            | 0.20           | 0.82         | 9.12           | 0.32          | 6.49   | 10.25           | 1.58          | 16.75      | 1.7                  |
| SD            | 0.04          | 0.38            | 0.02           | 0.03         | 0.65           | 0.02          | 0.43   | 0.70            |               | 1.13       |                      |
| Closed disc   |               |                 |                |              |                |               |        |                 |               |            |                      |
| florets**     | 0.48          | 4.67            | 0.14           | 0.66         | 9.24           | 0.25          | 5.29   | 10.16           | 1.92          | 15.45      | 1.5                  |
| SD            | 0.02          | 0.16            | 0.00           | 0.05         | 0.31           | 0.01          | 0.18   | 0.37            |               | 0.20       |                      |
| Receptacu-    |               |                 |                |              |                |               |        |                 |               |            |                      |
| lums**        | 0.16          | 1.45            | 0.05           | 0.20         | 2.49           | 0.07          | 1.66   | 2.76            | 1.66          | 4.42       | 0.4                  |
| SD            | 0.02          | 0.10            | 0.00           | 0.01         | 0.08           | 0.00          | 0.12   | 0.09            |               | 0.22       |                      |
| Leaves        | 0.03          | 0.46            | 0.01           | 0.05         | 1.48           | 0.04          | 0.49   | 1.57            | 3.18          | 2.06       |                      |
| SD            | 0.01          | 0.20            | 0.00           | 0.01         | 0.26           | 0.01          | 0.21   | 0.27            |               | 0.45       | 0.2                  |
| Steam         | 0.002         | 0.02            | 0.001          | 0.01         | 0.11           | 0.002         | 0.03   | 0.12            | 4.65          | 0.15       | 0.01                 |
| SD            | 0.001         | 0.01            | 0.000          | 0.00         | 0.02           | 0.001         | 0.01   | 0.03            |               | 0.03       | 0.01                 |
| Roots         | 0.012         | 0.08            | 0.002          | 0.02         | 0.17           | 0.003         | 0.10   | 0.19            | 1.92          | 0.28       | 0.02                 |
| SD            | 0.005         | 0.03            | 0.001          | 0.00         | 0.00           | 0.00          | 0.04   | 0.01            |               | 0.04       | 0.03                 |

<sup>\*</sup> with 3/4-opened disc florets

obtained, all 6 pyrethrins were detected (Fig. 3). The highest pyrethrin content was detected in the flower heads, with 3/4-open disc florets (Fig. 1c), which contained on average 1.2% (Fig. 3), maximally 1.4%, and minimally 1.0% pyrethrins of dry weight (data not shown). The chromatogram of the flower heads (Fig. 2b) is almost identical to the chromatogram for the pyrethrins standard (Fig. 2a). Although the content of the flower heads did not reach that of the highly productive pyrethrum clones from present-day production countries (Ravishankar et al., 1989; Rajasekaran et al., 1993; Ikahu et al., 1994; MacDonald, 1995; Hitmi et al., 2000; Morris et al., 2006), it is comparable with pyrethrins from conventional production in Kenya, Australia and India (Head, 1966; Keskitalo, 1999), with 0.1% to 1.8% pyrethrins of flower dry weight. Thus the Adriatic origin (Heywood et al., 2004), as a source of pyrethrum genetic variability, could still be of interest for a breeding program to select of high-producing clones.

#### **Biosynthesis of pyrethrins in various plant parts**

Different parts of the same flower head, separated into three parts - receptaculum, closed disc florets and open disc florets with ray florets - did not contain the same quantity of pyrethrins. The highest pyrethrins content was analysed in completely open and most mature disc florets with ray florets, which contained 1.7% pyrethrins of dry weight, less in closed disc florets, which contained 1.5% pyrethrins of dry weight, and least in the remaining part of the flower, the receptaculum, which contained only 0.4% pyrethrins of dry weight (Tab. 1). Leaves, stems and roots contained less pyrethrins than flower heads in both locations (Tab. 1). Leaves contained an average of 0.2%, steam an average of 0.01% and roots an average of 0.03% pyrethrins of dry weight and are not suitable for pyrethrins production. Besides the lower content of pyrethrins in plant parts other than the flower, they also contained a higher Pyl/Pyll ratio. The Pyl/Pyll ratio of the flower heads of pyrethrum was always lower than the ratio of other plant parts (Tab. 1). This confirms previous observations (Head, 1966).

<sup>\*\*</sup> isolated from flower heads

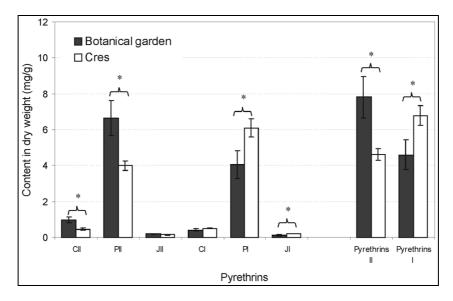


Fig. 3: The effect of location conditions on content levels of individual pyrethrins, pyrethrins I and II and total pyrethrins in flower heads of pyrethrum collected in the Botanical Garden of Ljubljana and on the island of Cres. Average (n = 5) contents of pyrethrins, standard deviations (SD) and statistically significant differences (t-test, \* denotes P < 0.05) are shown between samples from two locations. Legend: see Figure 2.

Sl. 3: Vpliv različnih rastiščnih razmer na vsebnost posameznih piretrinov, piretrinov I in II ter celokupnih piretrinov, v cvetnih glavicah bolhača, nabranega v Botaničnem vrtu v Ljubljani in na otoku Cresu. Prikazana je povprečna vsebnost posameznih piretrinov (n = 5), standardna deviacija (SD) in statistično značilne razlike (t-test, \* označuje P < 0.05) med vzorci obeh rastišč. Legenda: glej Sliko 2.

# Biosynthesis of pyrethrins in plants cultivated in regions with different climate conditions

Differences between plants cultivated under the different climate conditions of the island of Cres, Croatia, and the Botanical Garden in Ljubljana, had only a slight and insignificant influence on the total pyrethrin content in the flower heads (Fig. 3). Flower heads from island of Cres contained an average of 1.2% and flower heads from the Botanical Garden in Ljubljana contained an average of 1.1% pyrethrins of flower dry weight.

Differences between plants cultivated in different climate conditions of the island of Cres, Croatia, and the Botanical Garden in Ljubljana, however, did significantly influence the Pyl/Pyll ratio (Fig. 3), which is important for the insecticidal activity of pyrethrins and therefore affects the quality of the Pyrethrins extract (Jovetić & de Gooijer, 1995). Pyrethrins II were shown to have rapid knock-down ability and pyrethrins I slow killing effect (Jovetić & de Gooijer, 1995). The Pyl/Pyll ratio in flower heads from the island of Cres were noticeably higher (1.4) than the Pyl/Pyll ratio in flower heads from the Botanical Garden of Ljubljana (0.6) (Fig. 3). Ratios lower than 1 were observed in four of five samples from the Botanical Garden of Ljubljana, while in the remaining one this ratio increased to a level com-

parable to that observed in flower heads from Cres (data not shown). Beside the difference in environmental conditions, variations in Pyl/Pyll ratio may be caused by other factors, not examined in our study, such as slightly different stages of maturity at the time of flower sampling (Pattenden, 1970), slightly different management of flower samples or different cultivation conditions during plant growth on each site.

In conclusion, these data indicate that differences in location and climate conditions did not influence the total pyrethrin content in the flower heads, but did influence the Pyl/Pyll ratio. The pyrethrin content of flower heads from native species of the Northern Adriatic is comparable with content levels in the conventional production of pyrethrins, but did not reach the content levels of highly productive Pyrethrum clones from countries currently producing pyrethrins. Thus the Adriatic origin, as a source of Pyrethrum genetic variability, could be of potential interest in creating a breeding program for the selection of high-producing clones.

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# SEVERNOJADRANSKI BOLHAČ *(TANACETUM CINERARIIFOLIUM)* KOT POTENCIALNI VIR NARAVNIH INSEKTICIDOV

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#### **POVZETEK**

Bolhač Tanacetum cinerariifolium (Trevir.) Schultz-Bip. je najpomembnejši vir naravnih insekticidov piretrinov. Prispevek opisuje možnost pridobivanja piretrinov iz bolhača, s primarnih rastišč na kraških otokih in pobočjih priobalnega pasu Jadranskega morja nekdanje Jugoslavije in Albanije, nabranega na otoku Cresu in v Botaničnem vrtu v Ljubljani. V vseh vzorcih, analiziranih s tekočinsko kromatografijo visoke ločljivosti (HPLC), smo zaznali vseh 6 piretrinov. Največ piretrinov smo zaznali v cvetnih glavicah, ki so vsebovale povprečno 1.2% piretrinov v suhi masi vzorca. Razlike v rastiščnih razmerah obeh lokacij so malo in neznačilno vplivale na celokupno vsebnost piretrinov. Vsebnosti piretrinov v bolhaču, ki izvira iz naravnih rastišč severnega Jadrana, so primerljive z vsebnostjo piretrinov bolhača iz konvencionalne proizvodnje piretrinov, ki pa ne dosegajo vsebnosti visoko-produktivnih klonov bolhača, o katerih poročajo nekatere države sedanje proizvajalke piretrinov. Primarna rastišča priobalnega pasu Jadranskega morja, ki so vir genetske pestrosti bolhača, so zato še vedno zanimiva za programe žlahtnjenja in selekcijo visoko-produktivnih klonov.

Ključne besede: bolhač, Tanacetum cinerariifolium, piretrini, HPLC, severni Jadran

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# VEGETATION OF TALL RUSH SALTMARSHES (JUNCETEA MARITIMAE) AND SALTMARSH SCRUBS (ARTHROCNEMETEA FRUTICOSAE) ON THE SLOVENIAN SEACOAST

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#### **ABSTRACT**

Halophyte vegetation of Slovenian sedimentary seacoast was studied according to Braun-Blanquet method. 140 collected relevés were analysed by cluster analysis and five main clusters were separated. Relevés from the first, fourth and fifth clusters were further elaborated in this study. The Juncus maritimus-dominated tall rush saltmarshes of the class Juncetea maritimi were classified within two associations: Limonio-Puccinellietum represented hygrophilous stands, exposed to tide, while the association Juncetum maritimi-acuti was represented with more closed stands with higher species richness, thriving on rather drier sites. Within the saltmarsh scrubs of the class Arthrochemetea fruticosi, 3 associations, following the declining moisture/salinity gradient were recognized: Puccinellio-Arthrochemetum, Puccinellio-Halimionetum and Limonio-Artemisietum.

Key words: phytosociology, halophyte vegetation, classification, North Adriatic

# VEGETAZIONE A GIUNCO DI ACQUE SALMASTRE (JUNCETEA MARITIMAE) E PRATERIE ALOFILE (ARTHROCNEMETEA FRUTICOSAE) SULLA COSTA SLOVENA

#### SINTESI

La vegetazione alofila della costa marina sedimentaria in Slovenia è stata studiata secondo il metodo di Braun-Blanquet. Centoquaranta rilievi sono stati analizzati con l'aiuto della analisi numeriche e cinque grandi gruppi sono stati separati. I rilievi appartenenti al primo, quarto e quinto gruppo sono stati ulteriormente elaborati durante lo studio. La vegetazione di acque salmastre dominata da Juncus maritimus, della classe Juncetea maritime, è stata classificata in due associazioni: Limonio-Puccinellietum con banchi igrofili, esposti alla marea, e Juncetum maritimi-acuti con banchi più ristretti con una diversità di specie più alta, sprosperanti in siti piuttosto secchi. All'interno delle praterie alofile della classe Arthrocnemetea fruticosi, 3 associationi sono state riconosciute seguendo il gradiente umidità/salinità declinante: Puccinellio-Arthrocnemetum, Puccinellio-Halimionetum e Limonio-Artemisietum.

Parole chiave: fitosociologia, vegetazione alofila, classificazione, Adriatico settentrionale

#### INTRODUCTION

The Slovenian short seacoast is under heavy pressure of urbanization, leisure activities and industry (Port of Koper). Its special feature is the geological bedrock, being the calcareous sandstone - Eocene flysch substrate. This substrate almost perfectly matches the territory of Slovenia; only a small part extends to the Italian territory. Flysch substrate results in dense hydrological system above ground due to its impenetrable properties. Three larger streams/rivers have their mouths in the Gulf of Trieste: the Rižana, Strunjanski potok and Dragonja. Alluvial deposits in the mouths resulted in salt-marshes, where different vegetation types probably occurred before human influence. After the Roman period and afterwards these alluvial coastal regions have been either conversed to salt pans either drained (Darovec, 1992). The present situation is only a transitional stage in abandonment and creation of new habitats on the seacoast. The dynamics have always been fast and enabled assemblages of different vegetation types on the seacoast. Abiotic conditions - shallow coast and large tide area are in favour of assemblage of the various types of halophyte vegetation, probably similar to the natural types, developed before the human interference. The halophyte vegetation is interesting and important from the conservational perspective, as it is threatened by all factors mentioned above. Many coastal habitats with different types of halophyte vegetation are listed in Annexes of the EU Directive on the conservation of natural habitats and of wild fauna and flora (Council Directive 92/43/EEC, 1992).

Syntaxonomical classifications of the Slovenian halophyte vegetation are based on the relevés taken by M. Kaligarič in the years 1984-87 and in 1998 and 1999, published so far only for annual pioneer vegetation (Salicornietea) and Spartina maritima-dominated swards (Spartinetea) (Kaligarič & Škornik, 2006). Some of those relevés have been taken in consideration also within the last complete revision of the North Adriatic halophyte vegetation (Poldini et al., 1999). This revision considered the global revision of Mediterranean halophyte vegetation by Géhu et al. (1984), Rivas-Martínez (1990), Géhu & Biondi (1995, 1996), Mucina (1997) and Géhu (1999). Recent studies of halophyte vegetation, to the exclusion of Tyrrhenian district (Latium), have been made by Iberite & Frondoni (1997) and Frondoni & Iberite (2002). Both consider the already revised syntaxonomy.

The first phytosociological assessment of the North Adriatic halophyte vegetation was made much earlier by Béguinot (1941). The most profound research was carried out by Pignatti (1966) and followed by Fornaciari (1968). The Pignatti's syntaxonomical scheme (e.g. macroassociation "Limonietum venetum") was adopted also for Slovenian halophyte vegetation in some short contri-

butions about Sečoveljske soline (Kaligarič & Tratnik, 1981; Kaligarič & Wraber, 1988), Strunjan (Šajna & Kaligarič, 2005) and Škocjanski zatok (Kaligarič, 1997, 1998). The Pignatti's syntaxonomical units are also used for the threat status of halophyte flora and vegetation of the Slovenian seacoast (Kaligarič, 1996).

The elaboration of collected relevés from the Slovenian seacoast with classification methods and determination of major groups of vegetation (classes and orders) on the basis of classification methods and characteristic species has been carried out already by Kaligarič & Škornik (2006). The aim of this study was elaboration of the *Juncus maritimus*-dominated tall rush saltmarshes of the class *Juncetea maritimi* and the saltmarsh scrubs of the *Arthrocnemetea fruticosi* class.

#### **MATERIAL AND METHODS**

#### Study area

The Slovenian seacoast consists of flysch cliffs with scarce halophyte vegetation and sedimentary coast, mainly converted to salt pans or dried. In some parts, natural patches of coast still exist, while in other parts the sedimentary coast is even artificially enlarged (like soil deposits, etc.). In some parts (Sečoveljske soline), salt pans were abandoned and halophyte vegetation spread out. Locations (Fig. 1) were relevés have been sampled are: Sečoveljske soline (mouth of the Dragonja river, Fontanigge, Lera, San Giorggio channel), Strunjanske soline (Stjuža lagoon coast, salt pans), Škocjanski zatok, mud deposits and coastal grasslands near Sv. Katarina/Ankaran (Kaligarič & Škornik, 2006).

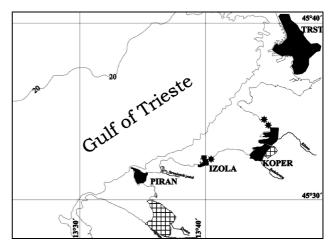


Fig. 1: Map of Slovenian seacoast with locations of the collected relevés of halophilous vegetation.

Sl. 1: Karta slovenske morske obale z lokalitetatmi popisov halofitne vegetacije. Mitja KALIGARIČ & Sonja ŠKORNIK: VEGETATION OF TALL RUSH SALTMARSHES (JUNCETEA MARITIMAE) AND SALTMARSH ..., 47–58

# **Sampling methods**

Using the standard Braun-Blanquet procedure (Braun-Blanquet, 1964; Westhoff & van der Maarel, 1973; Dierschke, 1994), relevés were taken during the years 1984–87 and 1998–1999. In the initial procedure, all the relevés were classified using the SYN-TAX 2000 software package (Podani, 2001). A hierarchical classification algorithm (Complete linkage, Eucledian distance) based on quantitative data was employed. From the resulting dendrogram (Fig. 2), three clusters were elaborated in previous study (Kaligarič & Škornik, 2006); all the other clusters were elaborated within this study. Division to classes and orders were based on main clusters, further subdivision to associations was carried out with the help of characteristic species (their frequencies and dominance) of the syntaxa.

Taxonomic nomenclature followed Martinčič et al. (1999) except for taxon *Halimione portulacoides*, syntaxonomic nomenclature followed Poldini et al. (1999).

# **RESULTS AND DISCCUSION**

#### Classification of the relevés

The 140 relevés were classified in 5 main clusters according to species composition and abundance, considering all the species of equal importance (Fig. 2). The most diverse was the first main cluster (No.1), which could be further divided into 7 sub-clusters (1A-1G). The first one (A, relevés 1-4) was represented by floristically poor Salsola soda dominated stands on dry trampled muddy soils with stones, where Elytrigia atherica and Atriplex prostrata indicate relatively dry conditions and lower salinity (Kaligarič & Škornik, 2006). This group of relevés was classified within the class Cakiletea maritimae (Kaligarič & Škornik, 2006). The third sub-cluster (1C, relevés 15-20) was characterized by dominance of grass Spartina maritima, and it was also elaborated in previous study (Kaligarič & Škornik, 2006). Other five sub-clusters (1B, 1D, 1F, 1G and part of 1E) were Juncus maritimus-dominated saltmarshes, relatively rich in various halophyte hygrophilous species. Among them were also halophytes such as Phragmites communis and characteristic species of the class Juncetea maritimae, Carex extensa and Plantago cornuti, which are not present in other halophilous vegetation types. These relevés (relevés 5-14 and 21-44) were classified within the class Juncetea maritimi (Kaligarič & Škornik, 2006).

The second main cluster (No. 2, relevés 45–67) and the third main cluster (No. 3, relevés 68–86), characterized by *Salicornia europaea*-dominated stands on mud-

flats or salt pans, were classified within the class *Thero-Salicornietea* and elaborated in the study by Kaligarič & Škornik (2006).

The fourth main cluster (No. 4, relevés 87–137) had many sub-clusters on a lower level of dissimilarity. The main characteristics were scarce presence of pioneer annual halophytes (Salicornia and Suaeda) and strong presence and dominance of halophilous scrubs: Arthrocnemum macrostachyum, Halimione portulacoides, Limonium angustifolium, Artemisia caerulescens, but also Puccinellia palustris, Inula crithmoides, and Aster tripolium. This perennial halophilous vegetation of relatively dry soil was spread predominately on abandoned salt pans or higher levels (above the tidal area). It was classified within the class Arthrocnemetea fruticosi (Kaligarič & Škornik, 2006).

The fifth main cluster with only 3 relevés (No. 5, relevés 138–140) represented *Juncus maritimus*-dominated salt grasslands near Sv. Katarina (Ankaran), which was classified within the class *Juncetea maritimi* (Kaligarič & Škornik, 2006), despite the presence of some non-halophytic species and its particular species richness.

Within this study, the clusters representing classes Arthocnemetea, Juncetea and Cakiletea will be further elaborated.

The syntaxa were determined mainly on the basis of cluster analysis (Fig. 2), but in some floristically very impoverished relevés the dominance of characteristic species was weighted over the automatic classification given by the expert system.

#### Vegetation of saltmarsh scrubs

Syntaxonomical classification of halophilous vegetation in the Mediterranean was very variable - from one class (Salicornietea) up to seven classes (Poldini et al., 1999). On the basis of different studies across the Mediterranean, European coastal halophyte vegetation is classified in three classes. The vegetation of perennial halophytes - mainly succulent chamaephytes and nanophanerophytes, which form more or less densely vegetated saltmarsh scrubs, is classified as Arthrocnemetea fruticosi. The distribution of this class of vegetation is not limited to the Atlantic and Mediterranean coasts, but extends to the continental halophilous vegetation of North Africa and Asia. The order Arthrocnemetalia fruticosi has a Mediterranean and Atlantic distribution. Alliance Arthrocnemion fruticosi is the Arthrocnemum fruticosum-dominated vegetation within the area mentioned above.

Dissimilarity

# 4 15 16 CAK JUNC SP JUNC .....32 ΑF JUNC 3 .....87 AF 5

Fig. 2: Dendrogram: the result of hierarchical classification analysis for 104 relevés of halophilous vegetation of the Slovenian seacoast.

**Legend:** black circles – ass. Salsoletum sodae; pink circles – ass. Juncetum maritime-acuti; blue circles – ass. Limonio narbonensis-Puccinellietum palustris; orange circles – ass. Puccinelio festuciformis-Arthrocnemetum perennis; green circles – ass. Limonio narbonensis-Artemisietum coerulescentis; violet circles – ass. Puccinellio festuciformis-Arthrocnemetum fruticosi.

Sl. 2: Dendrogram: rezultat hierarhične klasifikacije 104 popisov halofitne vegetacije ob slovenski obali.

**Legenda: črni krogci – as.** Salsolo kali-Cakiletum maritimae; **rožnati krogci – as.** Juncetum maritime-acuti; **modri krogci – as.** Limonio narbonensis-Puccinellietum palustris; **oranžni krogci – as.** Puccinelio festuciformis-Arthrocnemetum perennis; **zeleni krogci – as.** Limonio narbonensis-Artemisietum coerulescentis; **vijolični krogci – as.** Puccinellio festuciformis-Arthrocnemetum fruticosi.

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# Association Limonio narbonensis-Artemisietum coerulescentis Horvatić (1933) 1934 corr. Gehu et Biondi 1996 (Tab. 1)

This is the driest association in the humidity/salinity gradient of perennial scrubs, with really dense vegetation cover, and highest species-richness in this series of syntaxa. The presence of halotolerant species, which penetrate from marginal areas outside salty soils, is also characteristic. It was described from Dalmatia by Horvatić in 1934 and registered many times in NE Italy (e.g. Poldini et al., 1999). Halophytes with less extreme adaptations are characteristic of these stands, which could be explained with less extreme site conditions. The summer dry period is also characteristic.

On the Slovenian seacoast, this association occurs predominately in abandoned salt pans, on elevated sites, banks, and closed muddy surfaces with only temporary inundation.

# Association *Puccinellio festuciformis-Halimionetum portulacoidis* Géhu, Biondi, Géhu Franck *et* Costa 1992 (Tab. 2)

This association was described by Ferrari et al. (1985) from Italy. It is characterized by dominance of Halimione portulacoides. Its position along the humidity/salinity gradient is the middle one, between the three associations of the order. It covers smaller surfaces, on better drained soils that the previous association. It develops on margins of abandoned salt pans; bottoms of banks, the presence of rougher granulation of soils (e.g. smaller parts of stony banks in salt pans) seem to promote the dominance of Halimione portulacoides. The vegetation is not as dense as in the previous association. This association had been previously at least partly classified as a subassociation (halimionetosum) of the macroassociation "Limonietum venetum", described by Pignatti (1952). This macroassociation was later divided into different units by Géhu et al. (1984) due to scientific and technical (nomenclatural) arguments.

Tab. 1: Analytical table of the association Limonio narbonensis-Artemisietum coerulescentis Horvatić (1933) 1934 corr. Géhu et Biondi 1996.

Legend: CI – Arthrocnemetea fruticosi Br.-Bl. et R. Tx. 1943 corr. O. Bolós 1967; O – Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. O. Bolós 1967; A – Arthrocnemion fruticosi Br.-Bl. 1931 corr. O. Bolós 1967.

Tab. 1: Analitična tabela asociacije Limonio narbonensis-Artemisietum coerulescentis Horvatić (1933) 1934 corr. Géhu et Biondi 1996.

**Legenda:** Cl – Arthrocnemetea fruticosi Br.-Bl. et R. Tx. 1943 corr. O. Bolós 1967; O – Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. O. Bolós 1967; A – Arthrocnemion fruticosi Br.-Bl. 1931 corr O. Bolós 1967.

|                                   |        |        |     | 110 | 445 | 446 | 1   | 110 | 440 | 400 | 101 | 100 |         |
|-----------------------------------|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| Relevé number                     | 87     | 89     | 99  | 112 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 |         |
| Original relevé number            | 54     | 65     | 62  | 55  | 56  | 57  | 58  | 59  | 60  | 64  | 61  | 63  | Fr. (%) |
| Diagnostic species of the associa | tion   |        |     |     |     |     |     |     |     |     |     |     |         |
| Cl Limonium angustifolium         | 1      | 2      | +   | 2   | 1   | +   |     | 3   | 2   | 3   | 2   | 2   | 92      |
| Cl Artemisia coerulescens         | 2      | 1      | 2   |     | 3   | 3   | 3   | 2   | 2   | 1   | 2   | 3   | 92      |
| Diagnostic species of higher syn  | taxono | mic un | its |     |     |     |     |     |     |     |     |     |         |
| O Halimione portulacoides         | 4      | 2      | 3   | 2   | 3   | 1   | +   | +   | +   | 1   | +   | +   | 100     |
| Cl, A Inula crithmoides           | +      | +      | 1   | 3   | +   |     | +   | +   | 1   | 1   | +   | 1   | 92      |
| Cl, O Sarcocornia fruticosa       | 1      | 1      | 3   | 2   |     |     |     |     |     |     |     | +   | 42      |
| Others                            |        |        |     |     |     |     |     |     |     |     |     |     |         |
| Aster tripolium                   | +      |        | 1   |     | +   | +   | +   | +   |     |     | 2   |     | 58      |
| Elytrigia atherica                | +      |        | +   |     |     |     | +   | +   |     | 1   | +   |     | 50      |
| Suaeda maritima                   | 1      |        |     |     | +   | +   | +   | 1   |     |     |     | +   | 50      |
| Puccinellia palustris             | 2      |        |     | 1   |     |     |     |     | +   |     | 1   | 2   | 42      |
| Elytrigia elongata                |        |        | +   |     |     |     |     |     |     | +   | +   |     | 25      |
| Melilotus albus                   |        | +      |     |     |     |     |     |     |     | +   |     |     | 17      |
| Dactylis glomerata                |        |        | +   |     |     |     |     |     |     | +   |     |     | 17      |
| Arthrocnemum                      |        |        |     |     |     |     |     |     |     |     |     |     |         |
| macrostachyum                     |        |        |     |     |     | ٠   |     |     | +   |     |     | •   | 8       |
| Aster lynosiris                   |        |        |     |     |     | •   |     |     | •   | +   |     |     | 8       |
| Atriplex prostrata                |        |        | +   |     |     |     |     |     |     |     |     |     | 8       |
| Lotus corniculatus                |        |        |     |     |     |     |     |     |     | +   |     |     | 8       |
| Phragmites australis              | +      |        |     |     |     | •   |     |     | •   |     |     |     | 8       |
| Salicornia europaea               |        |        |     |     |     |     |     |     |     |     |     | +   | 8       |

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**Tab. 2: Analytical table of the association** Puccinelio festuciformis-Halimionetum portulacoidis **Géhu, Biondi. Géhu Franck** et **Costa 1992.** 

Legend: CI – Arthrocnemetea fruticosi Br.-Bl. et R. Tx. 1943 corr. O. Bolós 1967; O – Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. O. Bolós 1967; A – Arthrocnemion fruticosi Br.-Bl. 1931 corr O. Bolós 1967.

Tab. 2: Analitična tabela asociacije Puccinelio festuciformis-Halimionetum portulacoidis Géhu, Biondi. Géhu Franck et Costa 1992.

**Legenda:** Cl – Arthrocnemetea fruticosi **Br.-Bl.** et **R. Tx. 1943 corr. O. Bolós 1967; O** – Arthrocnemetalia fruticosi **Br.-Bl. 1931 corr. O. Bolós 1967; A** – Arthrocnemion fruticosi **Br.-Bl. 1931 corr O. Bolós 1967.** 

| Relevé number                      | 88   | 90  | 91    | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 108 |         |
|------------------------------------|------|-----|-------|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| Original relevé number             | 72   | 73  | 75    | 76 | 83 | 77 | 79 | 80 | 78 | 82 | 71  | 66  | 67  | 68  | 74  | 69  | 81  | 70  | Fr. (%) |
| Diagnostic species of the associat | ion  |     |       |    |    |    |    |    |    |    |     |     |     |     |     |     |     |     |         |
| O Halimione portulacoides          | 3    | 3   | 4     | 4  | 3  | 3  | 2  | 2  | 3  | 3  | 2   | 4   | 3   | 3   | 3   | 2   | 2   | 2   | 100     |
| Puccinellia palustris              | 2    | +   | +     | +  | +  | +  | +  | +  | •  | 1  | +   | +   | +   | 1   | +   | +   |     | +   | 89      |
| Diagnostic species of higher synta | xono | mic | units |    |    |    |    |    |    |    |     |     |     |     |     |     |     |     |         |
| Cl, O Sarcocornia fruticosa        | 2    | 1   | +     | 1  | 1  | +  | +  | +  | +  |    | 2   | 3   | 3   | 2   | 2   | 2   | 2   | 3   | 94      |
| Cl Limonium angustifolium          | +    | 1   | 1     | +  | +  | +  | +  | 1  | +  |    | +   | 1   | +   | +   | +   | 1   | 1   | +   | 94      |
| Cl Artemisia coerulescens          | 1    |     | +     | +  |    | +  | •  |    |    |    | 1   |     |     |     |     |     |     | +   | 33      |
| A Inula crithmoides                | •    | •   | +     | ٠  | •  | +  | •  | •  | •  |    | +   |     |     |     |     |     |     | +   | 22      |
| Others                             |      |     |       |    |    |    |    |    |    |    |     |     |     |     |     |     |     |     |         |
| Aster tripolium                    | +    | •   |       | ٠  | •  | +  | •  | +  | +  | +  | +   |     |     | +   |     | +   |     | +   | 50      |
| Elytrigia atherica                 | +    | +   | +     | +  | •  | •  | +  | •  | •  | 1  | +   |     |     |     | +   | +   |     |     | 50      |
| Suaeda maritima                    |      |     |       |    | +  | +  | +  | +  | 2  | +  | +   | •   | 1   | +   | •   |     |     |     | 50      |
| Arthrocnemum macrostachyum         | 1    |     |       |    | +  | •  |    |    | •  | 1  |     |     |     |     |     |     |     |     | 17      |
| Juncus maritimus                   |      |     |       |    |    |    |    |    | +  |    |     |     |     |     |     |     |     |     | 6       |

**Tab. 3: Analytical table of the association** Puccinellio festuciformis-Arthrocnemetum fruticosi (**Br.-Bl. 1928**) **Géhu 1976.** 

**Legend:** Cl – Arthrocnemetea fruticosi **Br.-Bl.** et **R. Tx. 1943 corr. O. Bolós 1967; O** – Arthrocnemetalia fruticosi **Br.-Bl. 1931 corr. O. Bolós 1967; A** – Arthrocnemion fruticosi **Br.-Bl. 1931 corr O. Bolós 1967.** 

Tab. 3: Analitična tabela asociacije Puccinellio festuciformis-Arthrocnemetum fruticosi (Br.-Bl. 1928) Géhu 1976. Legenda: Cl – Arthrocnemetea fruticosi Br.-Bl. et R. Tx. 1943 corr. O. Bolós 1967; O – Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. O. Bolós 1967; A – Arthrocnemion fruticosi Br.-Bl. 1931 corr O. Bolós 1967.

| Relevé number                | 28    | 33    | 34   | 35  | 36   | 37 | 107 | 109 | 110 | 111 | 113 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 |            |
|------------------------------|-------|-------|------|-----|------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|
| Original relevé number       | 90    | 91    | 92   | 93  | 94   | 95 | 107 | 101 | 108 | 104 | 89  | 84  | 88  | 85  | 105 | 109 | 86  | 87  | 96  | 98  | 99  | 106 | 97  | 100 | 102 | 103 | Fr.<br>(%) |
| Diagnostic species of the as | soc   | iatio | n    |     |      |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Cl, O Sarcocornia fruticosa  |       | 2     | 1    | 1   | 1    | 1  | 3   | 3   | 3   | 3   | 3   | 4   | 3   | 4   | 3   | 4   | 3   | 3   | 4   | 4   | 4   | 5   | 3   | 3   | 2   | 3   | 100        |
| Puccinellia palustris        | +     | +     |      |     |      |    |     |     | +   |     | 1   | +   | 1   | 1   | 1   | 1   | 1   |     | 1   |     |     |     | +   |     |     |     | 46         |
| Diagnostic species of higher | r syı | ntax  | onoi | mic | unit | 5  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Cl Limonium narbonense       | 4     | 4     | 4    | 3   | 3    | 3  | 1   | +   | +   | 1   | 4   | 2   | 2   | 3   | 3   | 3   | 3   | 3   |     |     |     | +   | +   | +   | +   | 1   | 88         |
| O Halimione portulacoides    | +     | +     | +    | 1   | 1    | 1  | 2   | 1   | 1   | 1   | 1   |     | +   |     |     |     |     |     |     |     |     |     |     | +   | +   | +   | 58         |
| A Inula crithmoides          |       |       | +    | +   | +    |    |     |     |     |     | 1   |     | +   | +   |     |     |     |     |     |     |     |     |     |     |     |     | 23         |
| Cl Artemisia caerulescens    |       |       |      | +   | +    |    |     |     |     |     | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 12         |
| Others                       |       |       |      |     |      |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Aster tripolium              | +     |       |      | +   | +    | +  |     |     |     |     |     | +   | +   | +   | +   |     | 1   | +   | +   |     | +   |     |     |     |     |     | 46         |
| Suaeda maritima              |       | 1     |      | +   | +    | +  |     |     |     | +   |     |     | +   |     |     | +   |     |     |     |     |     | +   |     |     |     | +   | 35         |
| Salicornia europaea          |       |       |      |     |      |    |     |     | +   | 1   |     |     |     |     | +   | +   |     |     |     |     | +   |     |     |     |     |     | 19         |
| Phragmites australis         |       | +     |      |     |      |    |     |     |     |     |     |     |     |     | +   |     |     | 1   |     |     | +   | +   |     |     |     |     | 19         |
| Elytrigia atherica           |       |       |      |     |      |    |     |     |     |     |     |     |     |     |     |     |     |     | +   |     |     |     |     |     |     |     | 4          |

**Puccinellio festuciformis-Arthrocnemetum fruticosi** (Br.-Bl. 1928) Géhu 1976 (Tab. 3)

This association, formerly considered as one of the most widespread subassociations ("arthrocnemetosum fruticosi") of the "Limonietum venetum", is extremely humid, for very long time inundated along the humid-

ity/salinity gradient of the three associations of the order. It has also the lower cover values. Water – predominately rainwater – stands in wintertime for longer periods, with no possibility to flow off. In the summer time it may dry up completely, especially within the abandoned salt pans of Sečoveljske soline, where one of the most abundant halophyte associations can be found. It is

characterized by dominance of *Sarcocornia fruticosa* and weak presence/cover of *Halimione portulacoides*. The rest of the floristic inventory is very similar to the previous association. Outside the Sečoveljske soline, patches of this association do not reach larger surfaces (a few square metres at the most). The association occurs on elevated sites in Strunjanske soline and Škocjanski zatok.

#### Vegetation of Juncus maritimus tall rush saltmarshes

The vegetation of coastal saltmarsh vegetation of the class *Juncetea maritimi* is characterized by the dominance of *Juncus maritimus* and other helophytes among halophytic species. These habitats are also supported by brackish water. The classis is further classified into the order *Juncetalia maritimi* and alliance *Juncion maritimi*, which is present on the North Adriatic coast with two suballiances: the (a) *Juncenion maritimi* is always characterized by dominance of *Juncus maritimus* and it is relatively rich with species of different syntaxa, from therophytes to scrubs, marshy halotolerant species to extreme succulents. Indicator species beside *J. maritimus* 

are *J. acutus*, which is currently not present on the Slovenian seacoast, *Plantago cornuti, Sonchus maritimus* and *Elytrigia elongata*. The suballiance *Puccinellienion festuciformis* is characterized by the strong presence of *Puccinellia palustris* (=*P. festuciformis*) and *Aster tripolium*. These stands are wet and frequently inundated.

# Association Juncetum maritimi-acuti Horvatić 1934 (suballiance Juncenion maritimi) (Tab. 4)

Juncus maritimus is absolutely dominant perennial in this association, which also forms main physiognomic aspect of these habitats, being in the permanent contact with sea or brackish water. One group of the relevés is more hygrophilous and characterized by Limonium angustifolium and Pucinellia palustris. It covers smaller surfaces at the shores of the channels or lagoons, sometimes at closed habitats in depressions with accumulating water. Another group of relevés is characterised by the natural saltmarsh at Sv. Katarina (Ankaran), where the conditions are drier and more species occur, including the rare ones, like Linum maritimum, Centaurium spicatum and Elytrigia elongata.

Tab. 4: Analytical table of the association Juncetum maritimi-acuti Horvatić 1934.

**Legend:** Cl – Juncetea maritimi **Br.-Bl. 1952 em. Beeftink 1965;** O – Juncetalia maritimi **Br.-Bl. 1931;** A – Juncenion maritimi **Géhu** et **Biondi 1995.** 

Tab. 4: Analitična tabela asociacije Juncetum maritimi-acuti Horvatić 1934

**Legenda:** Cl – Juncetea maritimi **Br.-Bl.** 1952 em. Beeftink 1965; O – Juncetalia maritimi **Br.-Bl.** 1931; A – Juncenion maritimi **Géhu** et **Biondi** 1995.

| Relevé number                 | 5     | 6      | 7    | 8     | 9   | 10  | 11  | 12  | 13  | 14  | 38  | 39  | 40  | 41  | 42  | 43  | 114 | 138 | 139 | 140 |            |
|-------------------------------|-------|--------|------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|
| Original relevé number        | 123   | 136    | 129  | 130   | 131 | 138 | 133 | 139 | 140 | 132 | 121 | 122 | 125 | 126 | 127 | 124 | 128 | 134 | 137 | 135 | Fr.<br>(%) |
| Diagnostic species of the a   | assoc | iation | )    |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Cl, O Juncus maritimus        | 3     | 3      | 3    | 4     | 4   | 4   | 3   | 3   | 4   | 5   | 4   | 3   | 3   | 2   | 2   | 2   | 1   | 4   | 3   | 4   | 100        |
| Diagnostic species of high    | er sy | ntaxo  | nomi | c uni | ts  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Cl Limonium angu<br>stifolium | 2     |        | +    | +     | +   | +   |     | 1   | 1   |     | 2   | 3   | 2   | 5   | 4   | 3   | 4   |     | +   | +   | 80         |
| Cl, O Puccinellia palustris   | 2     | 2      | +    | +     |     |     |     | +   |     |     | +   | +   |     | +   | +   | 2   | +   |     |     |     | 55         |
| Cl, O Aster tripolium         |       | 1      |      |       | +   | +   | +   |     | +   | +   |     |     |     | +   | 1   |     |     | +   |     | 2   | 50         |
| O Carex extensa               |       |        |      |       |     |     | +   |     |     |     |     |     | +   |     |     |     | +   | 1   | +   |     | 25         |
| A Plantago cornuti            |       |        |      |       |     |     |     |     |     |     |     |     |     |     | +   |     |     | 3   | 3   | 3   | 20         |
| A Sonchus maritimus           |       |        |      |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     | +   | +   | 10         |
| A Elytrigia elongata          |       |        |      |       |     | +   |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 5          |
| Others                        |       |        |      |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |            |
| Phragmites australis          |       | +      |      |       | +   |     | +   | +   | +   | 2   |     |     |     | +   |     | 2   |     | 1   | +   | 2   | 55         |
| Suaeda maritima               |       |        | +    |       |     |     | +   |     | +   | +   | +   |     | 1   |     |     | 1   |     |     |     |     | 35         |
| Inula crithmoides             |       |        |      |       |     |     |     |     |     |     | +   | +   | +   |     |     | +   | 2   |     |     | 1   | 30         |
| Halimione portulacoides       |       | +      |      |       |     | +   |     |     |     |     |     | +   | +   | +   |     |     | 1   |     |     |     | 30         |
| Artemisia caerulescens        |       |        |      |       |     |     | +   |     |     |     |     |     |     |     |     |     | +   | +   | +   | 1   | 25         |
| Elytrigia atherica            |       |        |      |       |     |     |     |     |     |     |     | +   |     |     |     |     |     | +   | 2   | 2   | 20         |
| Salicornia europaea           |       |        | +    |       |     |     |     |     |     | +   |     |     | +   |     | +   |     |     |     |     |     | 20         |
| Sarcocornia fruticosa         | +     |        |      |       |     |     | +   |     |     |     |     |     |     |     |     |     | +   |     |     |     | 15         |
| Bolboschoenus maritimus       |       |        |      |       |     |     | +   | +   |     |     |     |     |     |     |     |     |     |     | +   |     | 15         |
| Centaureum spicatum           |       |        |      | ٠     |     | +   |     |     |     | •   |     |     |     |     |     |     |     |     |     | +   | 10         |
| Linum maritimum               |       |        |      |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     | +   | 2   | 10         |
| Holoschoenus maritimus        |       |        |      |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     | +   |     | 5          |
| Dittrichia viscosa            |       |        |      |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     | +   |     | 5          |

**Tab. 5: Analytical table of the association** Limonio narbonensis-Puccinellietum palustris (**Pignatti 1966**) **Géhu** et **Scopp. 1984 in Géhu** et al. **1984.** 

**Legend:** Cl – Juncetea maritimi **Br.-Bl.** 1952 em. Beeftink 1965; O – Juncetalia maritimi **Br.-Bl.** 1931; subA – Puccinellienion festuciformis (**Géhu** et **Scopp.** 1984 in **Géhu**, **Scoppola**, **Caniglia**, **Marchiori** et **Géhu Franck** 1984) **Géhu** et **Biondi** 1995.

**Tab. 5: Analitična tabela asociacije** Limonio narbonensis-Puccinellietum palustris (**Pignatti 1966**) **Géhu** et **Scopp. 1984 in Géhu** et al. **1984**.

Legenda: Cl – Juncetea maritimi Br.-Bl. 1952 em. Beeftink 1965; O – Juncetalia maritimi Br.-Bl. 1931; subA – Puccinellienion festuciformis (Géhu et Scopp. 1984 in Géhu, Scoppola, Caniglia, Marchiori et Géhu Franck 1984) Géhu et Biondi 1995.

| Relevé number                         | 22    | 23    | 24  | 25  | 26  | 27  | 29  | 30  | 31  | 32  | 44  |         |
|---------------------------------------|-------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| Original relevé number                | 113   | 111   | 112 | 110 | 118 | 120 | 117 | 119 | 115 | 116 | 114 | Fr. (%) |
| Diagnostic species of the association |       |       |     |     |     |     |     |     |     |     |     |         |
| Cl Limoniumangustifolium              | +     | +     | +   | +   | 2   | 2   | 5   | 4   | 4   | 2   | 2   | 100     |
| Cl, O, subA Puccinellia palustris     | 3     | 5     | 4   | 3   | 4   | 3   | +   | 1   | 1   | 1   |     | 91      |
| Diagnostic species of higher syntaxor | nomic | units |     |     |     |     |     |     |     |     |     |         |
| Cl, O Aster tripolium                 | +     |       |     | +   | +   | +   | +   | +   | •   | •   | 4   | 64      |
| Cl, O Juncus maritimus                |       |       |     |     | 1   | +   | +   |     | +   | +   |     | 45      |
| O Carex extensa                       |       |       |     |     | •   |     | •   |     | •   | •   | +   | 9       |
| Others                                |       |       |     |     |     |     |     |     |     |     |     |         |
| Phragmites australis                  |       |       |     |     | +   | +   |     | +   |     |     | +   | 36      |
| Suaeda maritima                       |       |       |     | 1   | •   |     | •   |     | 1   | +   | +   | 36      |
| Salicornia europaea                   | +     |       |     |     | •   |     | +   |     | •   | +   | •   | 27      |
| Halimione portulacoides               |       | •     | 1   | •   |     | •   |     | •   |     |     |     | 9       |
| Spartina maritima                     |       |       |     |     | +   |     |     |     |     |     |     | 9       |

Association Limonio narbonensis-Puccinellietum palustris (Pignatti 1966) Géhu et Scopp. 1984 in Géhu et al. 1984 (suballiance Puccinellienion festuciformis) (Tab. 5)

This association is characterized by combined domination of *Puccinellia palustris* and *Limonium angustifolium*. It appears in belts along the channel banks, lagoon margins, with high level of salt or brackish water. These stands are exposed to tidal oscillations and strong nutrient flow. *Juncus maritimus* is present, but not in all relevés. This association is in contact with syntaxa of the class *Spartinetea* on one side and syntaxa of the class *Arthrocnemetea* on the other side. The morphology of the dominant *Limonium angustifolium* species is different from drier sites, inhabited by *Arthrocnemetea* vegetation: here *Limonium* is taller and bigger. This species reaches its ecological optimum in two quite different habitats, classified in two different phytosociological classes.

# **CONCLUSIONS**

The elaborated associations are presented in synoptic table (Tab. 6), where some *Salsola soda*-dominated stands are included and classified within the class *Cakiletea maritime*, provisionally treated in the rank of association *Salsoletum sodae*, due to the presence of only two *Cakiletea* characteristic species – *Salsola soda* and

Atriplex prostrata. This anthropogenic stands with very small surfaces on the stony seashore or at the tops of the muddy dikes within some salt basins are very poor in species composition and therefore very hard to classify. This classification in the synoptic table is provisional only and it will not be further discussed in this treatise.

We could summarize that the two classes Juncetea maritimae and Arthrocnemetea fruticosae indeed differ ecologically, but further delimitation of the lower syntaxa (associations, namely) is based mostly on frequency and coverage of the species, e.g. Arthrocnemum vs. Halimione vs. Artemisia within the Arthrocnemetea classis, and Limonium/Puccinellia vs. Juncus and typical Juncetea inventory. Characteristic species for the two classes of halophilous vegetation are distributed more or less across all five (six) associations. It is very difficult to draw conclusions only on the basis of species presence and cover. The "understanding" of vegetation assemblage is beyond the species combinations and numerical classifications: it should be accompanied with ecological data (salinity, water potential, soil properties, nutrients etc) and functional plant traits (Kaligarič & Škornik, 2006). For a better understanding of the classification of respective vegetation types, Poldini et al. (1999) incorporated structural data (life form and growth form of plants) into his treatise. For causal understanding, where pure classification is only a starting point, an ecophysiological and morphological approach is needed.

**Tab. 6: Synoptic table of the associations of the classes** Arthrocnemetea fruticosi, Juncetea maritimi **and** Cakiletea maritimae. **Values in the table correspond to the relative frequencies (in percentage) of the species in presented group of relevés.** 

**Legend:** Li-Ar: Limonio narbonensis-Artemisietum coerulescentis; **Pu-Ha:** Puccinellio festuciformis-Halimionetum portulacoidis; **Pu-Ar:** Puccinellio festuciformis-Arthrocnemetum fruticosi; **Ju ma:** Juncetum maritimi-acuti; **Li-Pu:** Limonio narbonensis-Puccinellietum palustris; **Sals:** Salsoletum sodae; **Di1-5:** diagnostic species of ass.; **Cl1:** Arthrocnemetea fruticosi; **Cl2:** Juncetea maritimi; **O1:** Arthrocnemetalia fruticosi; **O2:** Juncetalia maritimi; **subA2:** Puccinellienion festuciformis.

**Tab. 6: Sinoptična tabela asociacij razredov** Arthrocnemetea fruticosi, Juncetea maritimi **in** Cakiletea maritimae. **Vrednosti v tabeli ustrezajo frekvencam (v odstotkih) pojavljanja vrst v predstavljenih skupinah popisov.** 

Legenda: Li-Ar: Limonio narbonensis-Artemisietum coerulescentis; Pu-Ha: Puccinellio festuciformis-Halimionetum portulacoidis; Pu-Ar: Puccinellio festuciformis-Arthrocnemetum fruticosi; Ju ma: Juncetum maritimi-acuti; Li-Pu: Limonio narbonensis-Puccinellietum palustris; Sals: Salsoletum sodae; Di1-5: diagnostične vrste asociacij; Cl1: Arthrocnemetea fruticosi; Cl2: Juncetea maritimi; O1: Arthrocnemetalia fruticosi; O2: Juncetalia maritimi; subA2: Puccinellienion festuciformis.

|   | Association                               | Li-Ar      | Pu-Ha | Pu-Ar | Ju ma | Li-Pu | Sals |
|---|---|------------|-------|-------|-------|-------|------|
|   | Number of relevés                         | 12         | 18    | 26    | 20    | 11    | 4    |
| Diagnostic species of the associa       | ntions                                    |            |       |       |       |       |      |
| Di1,Di5(Cl1, Cl2)                       | Limonium angustifolium                    | 92         | 94    | 88    | 80    | 100   |      |
| Di1, (Cl1)                              | Artemisia caerulescens                    | 92         | 33    | 12    | 25    |       |      |
| Di2(O1)                                 | Halimione portulacoides                   | 100        | 100   | 58    | 30    | 9     |      |
| Di2,Di3,Di5(Cl2, O2, subA2)             | Puccinellia palustris                     |            | 89    | 46    | 55    | 91    | 33   |
| Di3(Cl1, O1)                            | Sarcocornia fruticosa                     | 42         | 94    | 100   | 15    |       |      |
| Di4(Cl2, O2)                            | Juncus maritimus                          |            | 6     |       | 100   | 45    |      |
| Cl1 Arthrocnemetea fruticosi Bı         | Bl. et R. Tx. 1943 corr O. Bolós          | 1967       |       |       |       |       |      |
|   | Inula crithmoides                         | 92         | 22    | 23    | 30    |       |      |
| Cl2 <i>Juncetea maritimi</i> BrBl. 19   | 52 em. Beeftink 1965                      |            |       | •     |       | •     | •    |
|   | Aster tripolium                           | 58         | 50    | 46    | 50    | 64    |      |
|   | Carex extensa                             |            |       |       | 25    | 9     |      |
|   | Plantago cornuti                          |            |       |       | 20    |       |      |
|   | Sonchus maritimus                         |            |       |       | 10    |       |      |
|   | Elytrigia elongata                        | 25         |       |       | 5     |       |      |
| Cl3 <i>Cakiletea maritimae</i> R. Tx. e | et Prsg. 1950                             | •          |       |       |       |       |      |
|   | Salsola soda                              |            |       |       |       |       | 100  |
|   | Atriplex prostrata                        |            |       |       |       |       | 33   |
| Thero-Salicornietea Pignatti ex         | Tx. In Tx. Et Oberdorfer 1958 cor         | r. Tx.1974 |       |       |       |       |      |
|   | Salicornia europaea                       | 8          |       | 19    | 20    | 27    | 33   |
|   | Suaeda maritima                           | 50         | 50    | 35    | 36    |       |      |
| Spartinetea maritimae (R. Tx. 19        | 061) Beeft., Géhu, Ohba <i>et R</i> . Tx. | 1971       |       |       |       | •     | •    |
|   | Spartina maritima                         |            |       |       |       | 9     |      |
| Others                                  | •   |            |       |       |       | •     | •    |
|   | Puccinellia palustris                     | 42         |       |       |       |       |      |
|   | Melilotus albus                           | 17         |       |       |       |       |      |
|   | Dactylis glomerata                        | 17         |       |       |       |       |      |
|   | Aster lynosiris                           | 8          |       |       |       |       |      |
|   | Atriplex latifolia                        | 8          |       |       |       |       |      |
|   | Lotus corniculatus                        | 8          |       |       |       |       |      |
|   | Arthrocnemum                              | 8          | 17    |       |       |       |      |
|   | macrostachyum                             |            |       |       |       |       |      |
|   | Elytrigia atherica                        | 50         | 50    | 4     | 20    |       | 66   |
|   | Phragmites australis                      | 8          |       | 19    | 55    | 36    |      |
|   | Bolboschoenus maritimus                   | <u> </u>   |       |       | 15    |       |      |
|   | Centaurium spicatum                       |            |       |       | 10    |       |      |
|   | Linum maritimum                           | ·          |       | •     | 10    |       |      |
|   | Holoschoenus maritimus                    | ·          |       | •     | 5     |       |      |
|   | Dittrichia viscosa                        | <u> </u>   |       |       | 5     |       | •    |

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#### **APPENDIX 1**

Synoptic table of studied vegetation types:

*Arthrocnemetea fruticosi* Br.-Bl. et R. Tx. 1943 corr. O. Bolós 1967 (=*Sarcocornietea fruticosae* Br.-Bl. et R. Tx. 1943 corr. Castroviejo et Cirujano 1980)

Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. O. Bolós 1967 (=*Sarcocornietea fruticosi* Br.-Bl. 1931 corr. Castroviejo et Cirujano 1980)

Artrhrocnemion fruticosi Br.-Bl. 1931 corr. O. Boloó 1967

- Limonio narbonensis-Artemisietum coerulescentis Horvatić (1933) 1934 corr. Géhu et Biondi 1996
- Puccinellio festuciformis-Halimionetum portulacoidis Géhu, Biondi, Géhu Franck et Costa 1992
- Puccinellio festuciformis-Arthrocnemetum fruticosi (Br.-Bl. 1928) Géhu 1976

Juncetea maritimi Br.-Bl. 1952 em. Beeftink 1965

Juncetalia maritimi Br.-Bl. 1931 Juncion maritimi Br.-Bl. 1931

Puccinellenion festuciformis (Géhu et Scopp. 1984 in Géhu, Scoppola, Caniglia, Marchiori et Géhu Franck 1984) Géhu et Biondi 1995

- Limonio narbonensis-Puccinellietum palustris (Pignatti 1966) Géhu et Scopp. 1984 in Géhu et al. 1984
- Juncenion maritimi Géhu et Biondi 1995
- Juncetum maritimi-acuti Horvatić 1934

Cakiletea maritimae R. Tx. et Prsg. 1950 Euphorbietalia peplis R. Tx. 1950 Thero-Suaedion splendentis Br.-Bl. 1931

• Salsoletum sodae Pignatti 1953

#### **APPENDIX 2**

Localities of relevés:

Table 1: **87, 89**: Sečovlje – Fontanigge, MTB: 0547/2; **99**: Ankaran, MTB: 0448/1; **112, 115, 116–119**: Sečovlje – Fontanigge, MTB: 0547/2; **120**: Ankaran, MTB: 0448/1; **121, 122**: Sečovlje – Fontanigge, MTB: 0547/2. Table 2: **88, 90**: Sečovlje – Fontanigge, MTB: 0547/2; **91**: Koper–Škocjanski zatok, MTB: 0448/3 and 0448/4; **92–97**: Strunjan, MTB: 0447/4; **98, 100, 101**: Sečovlje – Fontanigge, MTB: 0547/2; **102, 103**: Strunjan, MTB: 0447/4; **104–106**: Sečovlje – Fontanigge, MTB: 0547/2; **108**: Koper – Škocjanski zatok, MTB: 0448/3 and 0448/4.

Table 3: 28, 33–36: Sečovlje – Fontanigge, MTB: 0547/2; 37: Strunjan, MTB: 0447/4; 107, 109: Sečovlje – Fontanigge, MTB: 0547/2; **110**: Strunjan, MTB: 0447/4; **111, 113, 123–127**: Sečovlje – Fontanigge, MTB: 0547/2; **128**: Koper – Škocjanski zatok, MTB: 0448/3 and 0448/4; **129, 130–133**: Koper – Škocjanski zatok, MTB: 0448/3 and 0448/4; 134: Strunjan, MTB: 0447/4; 135: Sečovlje – Fontanigge, MTB: 0547/2; 136: Strunjan, MTB: 0447/4; **137**: Sečovlje – Fontanigge, MTB: 0547/2. Table 4: 5, 6, 7: Sečovlje – Fontanigge, MTB: 0547/2; 8: Strunjan, MTB: 0447/4; 9: Sečovlje – Fontanigge, MTB: 0547/2; 10, 11: Ankaran, MTB: 0448/1; 12: Strunjan, MTB: 0447/4; 13: Sečovlje – Fontanigge, MTB: 0547/2; **14**: Koper – Škocjanski zatok, MTB: 0448/3 and 0448/4; 38: Sečovlje - Fontanigge, MTB: 0547/2; 39: Koper -Škocjanski zatok, MTB: 0448/3 and 0448/4; 40, 41: Sečovlje – Fontanigge, MTB: 0547/2; 42, 43: Koper – Škocjanski zatok, MTB: 0448/3 and 0448/4; **114**, **138**: Sečovlje - Fontanigge, MTB: 0547/2; 139, 140: Ankaran, MTB: 0448/1.

Table 5: **22–27**, **29**, **30–32**, **44**: Sečovlje – Fontanigge, MTB: 0547/2.

Mitja KALIGARIČ & Sonja ŠKORNIK: VEGETATION OF TALL RUSH SALTMARSHES (JUNCETEA MARITIMAE) AND SALTMARSH ..., 47–58

# VEGETACIJA OBMORSKIH MOČVIRIJ Z OBMORSKIM LOČKOM (JUNCETEA MARITIMAE) IN HALOFITNIH TRAJNIC (ARTHROCNEMETEA FRUTICOSAE) NA SLOVENSKI OBALI

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#### **POVZETEK**

Na kratki slovenski morski obali prevladujejo flišni klifi, kjer je halofitna vegetacija zelo slabo razvita. Zelo raznolika pa je halofitna vegetacija na muljastih sedimentih, tako naravnih kot antropogenih ali tistih v opuščanju (soline). Med leti 1984-87 in 1998-1999 je bilo zbranih 140 popisov halofitne vegetacije po standardni Braun-Blanquetovi metodi. V pričujočem članku smo podrobneje analizirali in predstavili tiste popise, ki zajemajo vegetacijo razredov halofitnih trajnic- grmičkov in polgrmičkov – Arthrocnemetea fruticosae in obmorskega ločkovja – Juncetea maritimi-acuti. Ob pomoči značilnih in dominantnih vrst v popisih smo identificirali 5 združb, in sicer v okviru prvega razreda tri združbe, ki sledijo gradientu vlažnosti/slanosti od najbolj suhih habitatov do najbolj zalitih v tem vrstnem redu: Limonio narbonensis-Artemisietum coerulescentis, Puccinellio festuciformis-Halimionetum portulacoidis in Puccinellio festuciformis-Arthrocnemetum fruticosi. Prva asociacija je najbolj sušna, dvignjena od vod, pojavlja se manj ekstremnih halofitov, značilna je vrsta Artemisia caerulescens. Sestoji so precej sklenjeni in vrstno pestri. V drugi je dominantna vrsta Halimione portulacoides, vegetacija je manj sklenjena, vrstno revnejša in zaseda manjše površine. V tretji asociaciji absolutno prevladuje vrsta Sarcocornia fruticosa, ki je najbolj namočena in ima najmanj sklenjeno vegetacijo. Za morska močvirja z vrsto Juncus maritimus (J. acutus manjka!) sta značilni dve asociaciji. Sestoji asociacije Limonio narbonensis-Puccinellietum palustris so razviti v pasovih ob obalah kanalov, lagun, v slani ali brakični vodi, so precej namočeni oziroma izpostavljeni plimovanju in dotoku hranil. Asociacijo označuje dominantnost vrst Limonium angustifolium ter Puccinellia palustris in je vrstno razmeroma revna. Asociacija Juncetum maritimi-acuti je vrstno pestrejša, označuje pa jo dominantnost obmorskega ločka. Nekaj popisov s prevladujočo vrsto Salsola soda je bilo klasificiranih kot Salsoletum sodae, ki sodi v razred Cakiletea maritimae. Sintezna tabela pokaže razlikovanje asociacij glede na dominantne vrste ter obstoj značilnic 2 (3) razredov halofitne vegetacije, kar je za uvrščanje v višje sintaksone v malovrstnih halofitnih sestojih vedno problematično. Zato se upoštevajo tudi nekatere strukturne značilnosti rastlin (življenjska oblika in oblika rasti), za globlje razumevanje pa je treba poznati tudi okoljske parametre in funkcionalne značilnosti rastlin.

Ključne besede: fitosociologija, halofitna vegetacija, klasifikacija, severni Jadran

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# HETEROPTERA OF SLOVENIA, IV: PENTATOMOMORPHA I

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# **ABSTRACT**

Species of the superfamilies Aradoidea, Lygaeoidea and Pyrrhocoroidea occurring in Slovenia are listed and data on the examined specimens presented. Six species or subspecies are reported for Slovenia for the first time: Aradus pallescens frigidus Kiritshenko, 1913, Ischnocoris hemipterus (Schilling, 1829), Stygnocoris cimbricus (Gredler, 1870), Stygnocoris fuligineus (Geoffroy, 1785), Stygnocoris matocqi Péricart, 1993 and Rhyparochromus sanguineus (Douglas & Scott, 1868). As far as the last species is concerned, however, the author believes that it is only a synonym for Rh. phoeniceus. 9 species, previously reported for Slovenia, have been omitted from the list due to misidentifications or misinterpreted localities.

Key words: Heteroptera, Pentatomomorpha, Slovenia, fauna

# HETEROPTERA IN SLOVENIA, IV: PENTATOMOMORPHA I

#### SINTESI

L'articolo presenta la lista delle specie delle superfamiglie Aradoidea, Lygaeoidea e Pyrrhocoroidea ritrovate in Slovenia, nonché la descrizione degli individui esaminati. Sei specie o subspecie vengono segnalate in Slovenia per la prima volta: Aradus pallescens frigidus Kiritshenko, 1913, Ischnocoris hemipterus (Schilling, 1829), Stygnocoris cimbricus (Gredler, 1870), Stygnocoris fuligineus (Geoffroy, 1785), Stygnocoris matocqi Péricart, 1993 e Rhyparochromus sanguineus (Douglas & Scott, 1868). L'autore è comunque convinto che nel caso dell'ultima specie sopra elencata si tratti di un sinonimo di Rh. phoeniceus. Nove specie, precedentemente segnalate per la Slovenia, sono state escluse dalla lista a causa di erronea identificazione o erronea interpretazione delle località.

Parole chiave: Heteroptera, Pentatomomorpha, Slovenia, fauna

gala leg.

#### **INTRODUCTION**

In the first part of a review of the Slovenian Pentatomomorpha species, the superfamilies Aradoidea (fam. Aradidae), Lygaeoidea (Lygaeidae, Piesmatidae, Berytidae) and Pyrrhocoroidea (Pyrrhocoridae) are presented. The classification used in the Catalogue of the Heteroptera of the Palaearctic region by Aukema & Rieger (2001) is followed. Thus, the family Lygaeidae is not split into several families as proposed by Henry (1997) according to the cladistic analysis, which showed Lygaeidae to be a paraphyletic group.

Species of the infraorder Pentatomomorpha are mostly herbivorous, but many of them feed predominantly on seeds, which have high nutrition value. Of the species presented in this part, only Geocorinae and some Rhyparochrominae are predatory. Some species of Aradidae feed on polyporaceous fungi, while other Aradidae suck the phloem sap of trees.

The first work treating Slovenian fauna by Scopoli (1763) listed more than 20 species of Pentatomomorpha (the identity of a few is not clear). He was the first to describe *Spilostethus pandurus* (Scopoli, 1763), *Spilostethus saxatilis* (Scopoli, 1763) and *Beosus maritimus* (Scopoli, 1763) among others. His description of *Cimex abietis* most probably refers to *Gastrodes abietum* Bergroth, 1914 and not to *Eremocoris abietis* (Linnaeus, 1758).

The fauna around the border town of Gorica (Gorizia) was dealt by Montandon (1886), Horváth (1887) and Reuter (1888). Eberstaller (1864) treated fauna of Styria and published a locality now in Slovenia. The fauna of the coastal region was dealt by Gräffe (1911). Most of his localities are now in Italy, and some in Slovenia.

Gogala & Moder (1960) published the first faunistic work dealing with the entire territory of Slovenia. Later lists of species and additions to them were published by Gogala & Gogala (1986, 1989, 1994), Gogala (1991, 1996) and Protić (2001). Several records are scattered in various works by other heteropterists.

For the present contribution, all the material kept in the Slovenian Museum of Natural History was checked and identifications proven or corrected. The exact localities of the examined specimens are published herewith mostly for the very first time.

# **LIST OF SPECIES**

# ARADIDAE **Aneurinae**

Aneurus avenius (Dufour, 1833)

Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 3. 6. 1949, Staudacher leg. Grosuplje, Taborska jama ok., VL78, 30. 8. 1981, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 14. 4. 1984 under bark of a *Malus* tree, A. & M. Gogala leg. Slavnik, VL14, 2. 6. 1984, A. & M. Gogala leg. Istra: Sečovlje, UL93, 3. 7. 1983, A. & M. Gogala leg. Kras: Dutovlje, Krajna vas, VL06, 17. 11. 1996, M. Go-

Prekmurje: Benica, Murska šuma, XM15, 22. 5. 2001, A. & M. Gogala leg.

Cerkniško jezero: Dolenja vas, VL47, 7. 5. 1995, S. Brelih leg.

Prekmurje: Muriša, XM25, 10. 4. 1997, S. Brelih leg. Bela krajina: Semič, WL15, 30. 4. 1983, V. Furlan leg.

#### **Aneurus laevis** (Fabricius, 1775)

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986; Heiss, 2006: Ponikva pri Žalcu, Studence (WM12) Specimens examined:

Ljubljansko barje: Log, Lukovica, VL59, 18. 9. 1983, A. & M. Gogala leg.

Brezje pri Dobrovi, VL49, 15. 6. 1986, M. Zdešar leg.

#### **Aradinae**

#### Aradus aterrimus Fieber, 1864

Gogala & Moder, 1960: Kočevje; Gogala & Gogala, 1986

Specimen examined:

Gottschee (= Kočevje), VL85, 6. 6. 1927, Staudacher leg.

#### Aradus betulae (Linnaeus, 1758)

Gogala & Moder, 1960: Mavrlen; Gogala & Gogala, 1986

Specimen examined:

Bela krajina: Meierle (= Mavrlen), WL14, 21. 5. 1933, Staudacher leg.

#### Aradus cinnamomeus Panzer, 1806

Gogala & Moder, 1960: Ljubljana, Pokojišče, Dobrova; Gogala & Gogala, 1986, 1989, 1994; Heiss, 2006: Dragonja, Stena

Specimens examined:

Pokojišče, VL58, 5. 5. 1929, 27. 4. 1930, 22. 5. 1932, Staudacher leg.

Laibach (= Ljubljana), 8. 8. 1937, Staudacher leg. Slavnik, VL14, 2. 6. 1984, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, 11. 7. 1987, A. & M. Gogala leg.

Istra: Dragonja, Stena, UL93, 30. 8. 1989, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 4. 2. 1990, A. & M. Gogala leg.

Črni kal, VL14, 13. 3. 1991, A. & M. Gogala leg.

Sečovlje, Sv. Onofrij, UL93, 28. 2. 1992, A. & M. Gogala leg.

Rakov Škocjan, VL47, 17. 8. 2001, A. Gogala leg.

# Aradus conspicuus Herrich-Schaeffer, 1835

Aradus dilatatus Dufour, 1845

Aradus crenatus auct. (non Say, 1832)

Gräffe, 1911: Trnovski gozd; Gogala & Moder, 1960: Šmarnogorska Grmada, Železniki, Bohinj, Pokojišče; Gogala & Gogala, 1986; Protić, 2001: Podčetrtek, E. Jaeger leg.; Heiss, 2006: Velika planina: Pl. Dovja raven (VM72), Kočevski Rog: Podstenice (WL06)

Specimens examined:

Železniki, VM32, 9. 7. 1933, Staudacher leg.

Toplice (= Dolenjske Toplice), WL06, 29. 7. 1934, Staudacher leg.

Šmarna gora, Grmada, južno pobočje, VM50, 13. 2. 1955, M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 15. 5. 1982 under bark of a *Fagus* stump, A. & M. Gogala leg.

Gorjanci: Koprivnik, WL26, 29. 5. 1995, T. Trilar leg. Kras: Brje pri Komnu, VL07, 4. 5. 1997, A. & M. Gogala leg.

Kraški rob: Zazid, Lipnik, 750 m, VL13, 29. 5. 2002, A. Gogala leg.

# Aradus corticalis (Linnaeus, 1758)

Eberstaller, 1864: Maribor; Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986

Specimens examined:

Laibach (= Ljubljana), 4. 5. 1930, 16. 8. 1934, 20. 9. 1942, Staudacher leg.

# Aradus depressus (Fabricius, 1794)

Gogala & Moder, 1960: Ljubljana, Otoče, Kot; Gogala & Gogala, 1986; Heiss, 2006: Kočevski Rog: Podstenice (WL06)

Specimens examined:

Kottal (= dolina Kot), VM14, 8. 6. 1935, Staudacher leg. Otoče, VM42, 14. 5. 1933, Staudacher leg.

Laibach (= Ljubljana), 16. 2. 1930, 20. 8. 1935, Staudacher leg.

Slavnik, VL14, 31. 5. 1981, A. & M. Gogala leg.

Bela krajina: Tanča gora, WL14, 26. 5. 1987, S. Brelih leg.

Pokojišče, VL58, 3. 6. 1988, S. Brelih leg.

Medvode, Goričane, VM51, 21. 5. 1990, 4. 6. 1990, F. Pohleven leg.

Vače, Zg. Slivna, VM80, 8. 5. 1990, S. Brelih leg. Ozeljan, Lijak, UL99, 8. 4. 1992, S. Brelih leg.

# Aradus erosus Fallén, 1807

Gogala & Moder, 1960: Ljubljana: Rožnik; Gogala & Gogala, 1986

Specimen examined:

Ljubljana: Rožnik, VM50, 29. 3. 1952, M. Gogala leg.

# Aradus lugubris Fallén, 1807

Gogala & Moder, 1960: Carniola, F. J. Schmidt leg.

#### Aradus obtectus Vásárhelyi, 1988

A. Gogala, 1996; Heiss, 2006: Kočevje, Ledenik (VL85) Specimen examined:

Kočevje, Strmec virgin forest, VL85, 25. 6. 1994 under bark of a dead *Abies alba* tree, A. & M. Gogala leg.

# Aradus pallescens Herrich-Schaeffer, 1840

Two subspecies, treated as species by Heiss (2001), but subspecies in the forthcoming work (Heiss, *pers. comm.*) occur in the region:

Aradus pallescens pallescens Herrich-Schaeffer, 1840 Montandon, 1886: Gorica

The record by A. Gogala (1991) refers to *A. p. frigidus*! *Aradus pallescens frigidus* Kiritshenko, 1913 (Fig. 1) Specimen examined:

Julijske Alpe: Zg. Radovna, 750 m, VM14, 10. 7. 1988, V. Furlan leg.

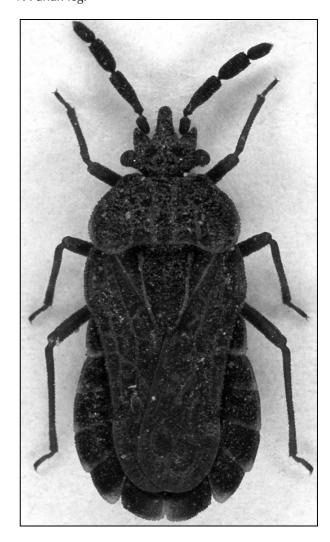


Fig. 1: Aradus pallescens frigidus Kiritshenko from Zg. Radovna.

Sl. 1: Aradus pallescens frigidus Kiritshenko iz Zg. Radovne.

#### Aradus somcheticus Kiritshenko, 1913

Heiss, 2006: Kočevski Rog: Podstenice (WL06)

#### Aradus versicolor Herrich-Schaeffer, 1835

Gogala & Moder, 1960: Ljubljana, Ig, Cerkno, Šklendrovec; Gogala & Gogala, 1986, 1989; Protić, 2001: Podčetrtek, E. Jaeger leg.; Heiss, 2006: Nova Gorica, Zagorje (UL99)

Specimens examined:

Laibach (= Ljubljana), 15. 6. 1932, Staudacher leg. Studenec – Ig, VL69, 25. 6. 1939, Staudacher leg. Šklendrovec, WM00, 29. 4. 1934, Staudacher leg. Cerkno, VM20, 1948, S. Brelih leg.

Pokojišče, VL58, 3. 6. 1988, S. Brelih leg.

Bohor: Plan. dom, WM30, 22. 6. 1989, S. Brelih leg. Makole, Pečke, WM53, 10. 7. 1993, T. Trilar leg. Polhograjsko hrib.: Topol, VM50, 30. 4. 1994, A. & M.

Gogala leg.

Vremščica, 880 m, VL26, 18. 5. 2002, A. Gogala leg. lg, Kremenica, VL68, 8. 5. 1998, S. Brelih leg. Luče, Raduha: Zavratnik, VM83, 27. 5. 1997, B.

Drovenik leg. Brezje pri Dobrovi, VL49, 15. 6. 1986, M. Zdešar leg. Ljubljana, Golovec: Orle, VL69, 27. 3. 1989, 10. 4.

1989, 21. 5. 1991, V. Furlan leg.
Topol, Ravnikar, VM50, 9. 6. 1991, V. Furlan leg.
Brežice, Terme Čatež, WI 48, 28, 4, 1998, V. Furlan leg.

Brežice, Terme Čatež, WL48, 28. 4. 1998, V. Furlan leg. Kras: Štanjel, Lukovec, VL07, 6. 6. 1988, R. Jelinčič leg.

# LYGAEIDAE Lygaeinae

#### Arocatus longiceps Stål, 1872

Gogala & Gogala, 1986

Specimens examined:

Istra: Portorož, Lucija, UL94, 2. 7. 1983 on *Platanus*, A. & M. Gogala leg.

Ljubljana: Šiška, VM60, 19. 12. 2002, 10. 6. 2004, 1. 11. 2004, 3. 11. 2004, S. Brelih leg.

# Arocatus melanocephalus (Fabricius, 1798)

Gogala & Moder, 1960: Mavrlen; Gogala & Gogala, 1986

Specimens examined:

Bela krajina: Meierle (= Mavrlen), WL14, 2. 5. 1933, Staudacher leg.

Istra: Dragonja, Stena, UL93, 2. 4. 2005 on *Cupressus*, A. Gogala leg.

# Arocatus roeselii (Schilling, 1829)

Horváth, 1887: Gorica; Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986

Specimens examined:

Laibach (= Ljubljana), 15. 7. 1929, 12. 4. 1930, Staudacher leg.

# Horvathiolus superbus (Pollich, 1781)

Lygaeus punctatoguttatus (Fabricius, 1781) Montandon, 1886: Gorica

#### Lygaeosoma sardeum Spinola, 1837

Lygaeosoma reticulatum (Herrich-Schaeffer, 1838) Montandon, 1886: Gorica; Gogala & Gogala, 1986, 1989, 1994; Péricart, 1998a: Hrastovlje Specimens examined:

Piran, UL84, 15. 4. 1979, A. & M. Gogala leg. Padna, UL93, 16. 6. 1984, 1. 2. 1997, A. & M. Gogala leg.

Dragonja, Stena, UL93, 1. 10. 1987 under *Sedum acre*, 6. 5. 2000, A. & M. Gogala leg.

Istra: Kubed, VL14, 16. 4. 1988, A. & M. Gogala leg. Kras: Brje pri Komnu, VL07, 9. 7. 1989, 7. 9. 1989, 26. 4. 2000, A. & M. Gogala leg., 4. 9. 2005, M. Gogala leg. Izvir Rižane, VL14, 18. 2. 1990, A. & M. Gogala leg. Osp, VL14, 18. 3. 1990, A. & M. Gogala leg. Dol. Branice: Čipnje, VL07, 13. 2. 2000, A. & M. Go-

Ljubljana, Golovec, VL69, 2. 6. 1989, V. Furlan leg. Strunjan, rt Ronek, UL94, 4. 9. 1998, V. Furlan leg.

#### Lygaeus equestris (Linnaeus, 1758)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Vižmarje, Šmarna gora, Jezersko, Bled, Bohinj, Notranjska, Soča; Gogala & Gogala, 1986, 1989, 1994
Specimens examined:

Veldes (= Bled), VM33, 3. 8. 1930, Staudacher leg. Wochein (= Bohinj), VM12, 20. 6. 1930, Staudacher leg. Kačiče – Divača, VL25, 22. 3. 1989, B. Drovenik leg. Bohinj: Ukanc, VM02, 3. 7. 1977, 5. 7. 1986, A. & M. Gogala leg.

Portorož, UL94, 7. 1. 1979, A. & M. Gogala leg. Portorož, Lucija, UL94, 2. 7. 1983, A. & M. Gogala leg. Črni kal, VL14, 15. 4. 1979, A. & M. Gogala leg., 2. 2. 1974, V. Furlan leg.

Strunjan, UL94, 22. 9. 1982, 16. 10. 1985, A. & M. Gogala leg.

Sečovlje, UL93, 2. 10. 1986, M. Gogala leg. Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg.

Istra: Koštabona, Supotski slap, VL03, 12. 10. 1988, A. & M. Gogala leg.

Kras: Vojščica, UL97, 6. 5. 1989, A. & M. Gogala leg. Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Brje pri Komnu, VL07, 2. 7. 1989, A. & M. Gogala leg. Osp, VL14, 18. 3. 1990, A. & M. Gogala leg. Padna, UL93, 1. 2. 1997, A. & M. Gogala leg. Dragonja, Pišine, UL93, 1. 2. 1997, A. & M. Gogala leg. Ljubljansko barje: Preserje, Sv. Ana, VL59, 18. 5. 1999,

A. Gogala leg. Hrpelje, VL15, 24. 6. 1999, S. Brelih leg. Bovec, Log čezsoški, UM82, 5. 7. 2001, S. Brelih leg.

Koper, Škocjanski zatok, VL04, 23. 4. 2002, S. Brelih

leg.

Razdrto, VL26, 16. 2. 1974, V. Furlan leg.

Črni kal, Socerb, VL15, 3. 5. 1980, V. Furlan leg.

Kras: Lipica, VL15, 30. 5. 1982, V. Furlan leg.

Polhograjsko hrib.: Grmada, VM40, 6. 5. 1984, V. Furlan leg.

Kamniška Bistrica, Konec, 1100 m, VM63, 18. 8. 1984, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 15. 4. 1984, V. Furlan leg.

Postojna, VL37, 10. 6. 1991, V. Furlan leg. Kozina, VL15, 22. 6. 1991, V. Furlan leg.

Trnovski gozd: Lokve, VL09, 27. 6. 1998, V. Furlan leg. Ljubljana, VL69, 16. 9. 1998, V. Furlan leg.

Strunjan, rt Ronek, UL94, 3. 9. 1998, V. Furlan leg.

# Melanocoryphus albomaculatus (Goeze, 1778)

Gogala & Moder, 1960: Ljubljana, Novo mesto; Gogala & Gogala, 1986

Specimens examined:

Stadtberg-Rudolfswert (= Novo mesto), WL17, 2. 10. 1932, Staudacher leg.

Laibach (= Ljubljana), 2. 10. 1937, Staudacher leg. Kras: Škocjan, Velika Dolina, VL25, 18. 4. 1992, A. Gogala leg.

Brje pri Komnu, VL07, 11. 10. 2003, 4. 7. 2004, A. Gogala leg.

#### Spilostethus pandurus (Scopoli, 1763)

Lygaeus militaris (Fabricius, 1775)

Scopoli, 1763: Circa Labacum (= around Ljubljana) & in Carniolia inferiore (= Dolenjska) – type localities; Montandon, 1886: Gorica; Gogala & Moder, 1960: Črni kal; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Istra: Strunjan, UL94, 16. 5. 1983, J. Carnelutti leg., 16. 10. 1985, A. & M. Gogala leg.

Portorož, Lucija, UL94, 2. 7. 1983, A. & M. Gogala leg. Portorož, Beli križ, UL84, 10. 10. 1984, M. Gogala leg. Piran, UL84, 3. 10. 1986, M. Gogala leg.

# Spilostethus saxatilis (Scopoli, 1763)

Scopoli, 1763: Carniola; Montandon, 1886: Gorica; Gogala & Moder, 1960; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Laibach (= Ljubljana), 20. 7. 1929, Staudacher leg. Medvode, Pirniče, VM51, 13. 2. 1977, A. & M. Gogala leg.

Bohinj: Ukanc, VM02, 24. 4. 1979, A. & M. Gogala leg. Ljubljansko barje: Ig, Kremenica, VL68, 1. 9. 1975, S. Brelih leg.

Velike Lašče, VL77, 25. 5. 1980, A. & M. Gogala leg. Ljubljana: Savlje, VM60, 13. 3. 1983, A. & M. Gogala leg.

Jezersko, VM64, 14. 8. 1983, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 7. 8. 1983, 19. 4. 1987, A. & M. Gogala leg.

Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala leg.

Ilirska Bistrica, Jelšane, VL43, 21. 6. 1983, M. Gogala leg.

Rovte, Medvedje brdo, VL39, 19. 5. 1985, A. & M. Gogala leg.

Rakitna, VL58, 22. 6. 1986, A. & M. Gogala leg.

Prekmurje: Petišovci, XM15, 13. 6. 1987, A. & M. Gogala leg.

Rakek, Unec, VL47, 14. 2. 1988, A. & M. Gogala leg. Idrija, Krekovše, VL19, 28. 6. 1988, M. Gogala leg. Julijske Alpe: Stara Fužina, Voje, VM13, 25. 9. 1988, A. & M. Gogala leg.

Istra: Koštabona, Supotski slap, VL03, 12. 10. 1988, A. & M. Gogala leg.

Dragonja, Stena, UL93, 30. 8. 1989, A. & M. Gogala leg.

Movraž, Movraška vala, VL13, 18. 5. 1990, A. & M. Gogala leg.

Dolina Raše: Griže, VL16, 21. 3. 1992, A. & M. Gogala leg.

Dolina Branice: Dolanci, VL17, 13. 2. 2000, A. & M. Gogala leg.

Breginj – Logje, UM72, 12. 6. 1997, S. Brelih leg. Sočerga, Šeki, VL13, 17. 6. 1999, S. Brelih leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, S. Brelih leg.

Obrov, Golac, VL24, 8. 6. 2000, S. Brelih leg. Rodik, 500 m, VL15, 7. 6. 2001, S. Brelih leg.

Logatec, VL48, 17. 2. 1974, V. Furlan leg.

Vipava, VL17, 27. 2. 1974, V. Furlan leg.

Muljava, VL88, 13. 4. 1980, 27. 5. 1982, V. Furlan leg. Krim, 600 – 1000 m, VL58, 8. – 17. 5. 1975, V. Furlan leg.

Kranjska Gora, VM04, 8. 11. 1975, V. Furlan leg. Nanos, 1248 m, VL27, 27. 3. 1977, V. Furlan leg. Kranj: Stražišče, VM42, 22. 4. 1977, V. Furlan leg. Škofja Loka, VM41, 10. 5. 1978, V. Furlan leg.

Gornji Ig, 600 m, VL68, 5. 6. 1982, 23. 5. 1987, V. Furlan leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg.

Novo mesto, Trška gora, WL17, 21. – 22. 5. 1983, V. Furlan leg.

Loški Potok: Retje, VL66, 7. 10. 1983, V. Furlan leg. Polhograjsko hrib.: Topol, VM50, 12. 11. 1983, V. Furlan leg.

Topol, Osredek, VM50, 30. 3. 1985, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 15. 4. 1984, V. Furlan leg.

Ratitovec, Prtovč, VM22, 10. 6. 1984, V. Furlan leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

Črni kal, Praproče, VL14, 12. 7. 1990, V. Furlan leg. Zasavje: Podkum, 740 m, WM00, 24. 5. 1989, V. Furlan leg.

Dol pri Ljubljani, VM70, 20. 5. 1988, V. Furlan leg. Radeče, Jagnjenica, WM00, 24. 5. 1990, V. Furlan leg. Ljubljana: Šmartno, VM60, 4. 5. 1988, V. Furlan leg. Ljubljana: Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Terme Čatež, WL48, 24. 4. 1998, V. Furlan leg.

#### **Tropidothorax leucopterus** (Goeze, 1778)

Lygaeus familiaris (Fabricius, 1781)

Horváth, 1887: Gorica; Gräffe, 1911: Tolmin; A. Gogala, 1996

Specimens examined:

Trenta, Alpinetum Julijana, VM03, 24. 9. 1993, A. & M. Gogala leg.

Bovec, Postaja B-Čela, 1100 m, UM83, 6. 8. 2000, S. Brelih leg.

Kras: Brestovica pri Komnu, UL97, 8. 9. 2002, A. & M. Gogala leg.

Kozje, Podsreda, Trebča Gorca, WM40, 9. 7. 1998, S. Brelih leg.

Kobariški Stol, 900 m, UM82, 10. 7. 2002, S. Brelih leg.

#### Orsillinae

#### *Nithecus jacobaeae* (Schilling, 1829)

Gogala & Moder, 1960: Julijske Alpe, Rateče, Kamniške pl., Golica; Gogala & Gogala, 1986, 1989 Specimens examined:

Kranjska Gora, Rateče, VM05, 1. 8. 1980, A. & M. Gogala leg.

Jelovica, VM32, 24. 8. 1980, A. & M. Gogala leg.

Pohorje: Areh, WM35, 24. 7. 1983, A. & M. Gogala leg. Pohorje: Lovrenška jezera, WM24, 23. 8. 1987, A. & M. Gogala leg.

Uršlja gora: Plešivec, VM94, 22. 8. 1987, A. & M. Gogala leg.

Julijske Alpe: Studorski preval – Vodnikova koča, VM13, 13. 9. 1987, A. & M. Gogala leg.

Komna – Vratca, VM02, 26. 8. 1990, A. & M. Gogala leg.

Košuta: Pl. Šija, 1530–1800 m, VM44, 20. 8. 1991, A. & M. Gogala leg.

Triglav, Vršič, 1600 m, VM04, 9. 8. 1991, E. Holzer leg. Mangart, Mangartsko sedlo, UM94, 2. 7. 1993, A. & M. Gogala leg.

Krvavec, Jezerca, VM62, 29. 7. 1992, A. & M. Gogala leg

Košuta: Tegoška gora, VM54, 14. 8. 1997, A. Gogala leg.

Menina pl.: Biba planina, VM82, 19. 7. 2006, A. & M. Gogala leg.

#### Nysius cymoides (Spinola, 1837)

Gogala & Gogala, 1986; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Julijske Alpe: Planina Pri jezeru, VM13, 20. 7. 1980, A. & M. Gogala leg.

Kranjska Gora, Rateče, VM05, 1. 8. 1980, A. & M. Gogala leg.

Kum, WM00, 26. 7. 1996, A. & M. Gogala leg.

#### Nysius graminicola (Kolenati, 1845)

Gogala & Moder, 1960: Ljubljana, Črni kal; Gogala & Gogala, 1986

Specimens examined:

Koper, Škocjanski zatok, VL04, 1. 7. 1979, 18. 5. 1980, A. & M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 7. 7. 2000, 22. 7. 2000, A. Gogala leg.

Sečovlje, Fontanigge, UL93, 20. 9. 1980, 21. 9. 1982, A. & M. Gogala leg., 18. 9. 2003, 13. 9. 2006, A. Gogala leg.

Strunjan, UL94, 30. 9. 1979, A. & M. Gogala leg. Portorož, UL94, 15. 10. 1986, A. & M. Gogala leg.

Portorož, Lucan, UL94, 12. 8. 1997, A. Kapla leg.

Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Padna, UL93, 1. 2. 1997, A. & M. Gogala leg.

Kras: Komen, Vale, UL97, 21. 10. 2000, A. & M. Gogala leg.

Istra: Trsek, VL03, 6. 8. 2001, A. Gogala leg.

Nova Gorica, Panovec, UL98, 13. 9. 2000, S. Brelih leg. Brestovica pri Komnu, Drenovce, UL97, 28. 6. 2005, S. Brelih leg.

Strunjan, rt Ronek, UL94, 3. 9. 1998, 6. 9. 1998, V. Furlan leg.

#### Nysius helveticus (Herrich-Schaeffer, 1850)

A. Gogala, 1991

Specimens examined:

Polhograjsko hrib.: Črni vrh, Pasja ravan, VM40, 15. 8. 1984, A. & M. Gogala leg.

Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris*, A. Gogala leg.

Gorjanci: Sv. Miklavž, WL26, 29. 8. 1990, V. Furlan leg.

#### Nysius senecionis (Schilling, 1829)

Gräffe, 1911: Tolmin; Gogala & Moder, 1960: Ljubljana, Lancovo; Gogala & Gogala, 1986, 1989

Specimens examined:

Radovljica, Lancovo, VM33, 4. 8. 1929, Staudacher leg. Laibach (= Ljubljana), 21. 9. 1928, 7. 10. 1928, Staudacher leg.

Prekmurje: Lendava, XM15, 6. 7. 1980, A. & M. Gogala leg.

Koper, Škocjanski zatok, VL04, 1. 7. 1979, 18. 5. 1980, A. & M. Gogala leg., 23. 4. 2002, S. Brelih leg.

Planina, VL47, 28. 6. 1982, M. Gogala leg.

Ajdovščina, VL18, 2. 8. 1985, A. & M. Gogala leg.

Istra: Strunjan, UL94, 16. 10. 1985, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 29. 7. 1990, A. & M. Gogala leg.

Nova Gorica, Panovec, UL98, 13. 9. 2000, S. Brelih leg.

# Orsillus depressus (Mulsant & Rey, 1852)

Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Brkini: Barka, VL25, 28. 7. 1984 on *Juniperus*, A. & M. Gogala leg.

Črni kal, VL14, 24. 10. 1984, 26. 10. 1985 on *Juniperus*, 17. 2. 1988, A. & M. Gogala leg.

Kras: Štorje, VL16, 26. 3. 1989, A. & M. Gogala leg. Istra: Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Brje pri Komnu, VL07, 9. 9. 2001 on *Juniperus*, A. & M. Gogala leg.

Hrpelje, Prešnica, VL14, 10. 5. 1999, S. Brelih leg. Prekmurje: Lendava, Dolga vas, XM16, 1. 11. 1999, S. Gomboc & D. Kofol leg.

Ljubljana, VM60, 3. 11. 1990, V. Furlan leg.

# Orsillus maculatus (Fieber, 1861)

Péricart, 2001a

Specimen examined:

Istra: Padna, UL93, 1. 2. 1997 on *Cupressus*, A. & M. Gogala leg.

# Ortholomus punctipennis (Herrich-Schaeffer, 1838)

Gogala & Moder, 1960: Dol pri Ljubljani, Kamniške pl. nad Okrešljem, along the Dragonja

Specimen examined:

Prekmurje: Muriša, 150 m, XM25, 15. 7. 2002, T. Trilar leg.

# Ischnorhynchinae

# Kleidocerys resedae (Panzer, 1797)

Gogala & Moder, 1960: Šentvid nad Ljubljano, Žirovnica; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Pokojišče, VL58, 11. 5. 1930, Staudacher leg.

Laibach (= Ljubljana), 17. 9. 1931, 10. 6. 1932, Staudacher leg.

Ljubljana: Šiška, VM50, 22. 4. 1981, A. Gogala leg., VM60, 15. 8. 2002, S. Brelih leg.

Janče, VM70, 7. 6. 1981, A. & M. Gogala leg.

Ljubljansko barje: Log, Lukovica, VL59, 18. 8. 1982, A. & M. Gogala leg.

Prekmurje: Moravci, WM97, 23. 7. 1983, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 4. 8. 1983, A. & M. Gogala leg., 25. 8. 2005 on *Calluna vulgaris*, A. Gogala leg.

Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala leg.

Brkini: Artviže, VL25, 28. 7. 1984 on *Betula*, A. & M. Gogala leg.

Kamniško-Savinjske Alpe: Krvavec, VM62, 25. 6. 1985, M. Gogala leg.

Sp. Brnik, VM61, 7. 9. 1988 on *Betula*, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Bloke: Godičevo, VL67, 2. 7. 2000, A. Gogala leg. Bela krajina: Drašiči, WL25, 3. 6. 1995, M. Gogala leg. Hrastnik, WM01, 9. 1997, A. Kapla leg.

Slovenj Gradec, Rahtelov vrh, WM05, 24. 9. 1999, M. Gogala leg.

Pokojišče, Zavrh, VL48, 9. 6. 2005, A. Gogala leg. Ljubljana: Rakovnik, VL69, 9. 4. 1991, V. Furlan leg. Ljubljana, Golovec, VL69, 19. 4. 1981, 4. 4. 1982, 23. 4. 1983, 23. 2. 1992, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 4. 5. 1985, V. Furlan leg.

Ig, Kremenica, VL68, 26. 9. 2002, S. Brelih leg. Ljubljana, Barje, VL69, 25. 8. 1980, V. Furlan leg. Vremščica, VL26, 11. 4. 1981, V. Furlan leg. Ljubljana, VM60, 11. 3. 1983, V. Furlan leg. Ljubljana: Nove Jarše, VM60, 9. 8. 1985, V. Furlan leg. Brezje pri Dobrovi, VL49, 20. 8. 1985, M. Zdešar leg. Kum, 1000 m, WM00, 30. 5. 1989, V. Furlan leg. Velika Preska, VL99, 28. 3. 1989, V. Furlan leg. Bela krajina: Preloka, WL23, 1. 8. 1990, V. Furlan leg.

#### Cyminae

#### Cymus aurescens Distant, 1883

Cymus obliquus Horváth, 1888

Gogala & Moder, 1960: Ljubljana, Dobrova; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Laibach (= Ljubljana), 31. 5. 1937, 2. 6. 1938, Staudacher leg.

Ljubljansko barje: Log, Lukovica, VL59, 2. 6. 1978, A. & M. Gogala leg.

Dobrova, VM50, 27. 5. 1979, A. & M. Gogala leg. Velike Lašče, VL77, 25. 5. 1980, A. & M. Gogala leg. Kamniško-Savinjske Alpe: Krvavec, VM62, 14. 6. 1981, A. & M. Gogala leg.

Osilnica, Plešce, Slovene side of the river, VL74, 27. 7. 1985, A. & M. Gogala leg.

Pomurje: Veržej, WM86, 13. 6. 1987, A. & M. Gogala leg.

Podčetrtek, Vonarje, WM41, 6. 8. 1996, A. Gogala leg. Gradišče pri Lukovici, VM71, 31. 7. 1996, A. Gogala leg.

Bizeljsko, Stara vas, WL59, 21. 4. 2000, A. Gogala leg. Moravče, Prikrnica, ob Drtijščici, VM71, 19. 5. 1997, S. Brelih leg.

Ljubljana, Lavrica, VL69, 20. 5. 1991, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 4. 5. 1984, 25. 4. 1990, 21. 5. 1991, V. Furlan leg.

Ljubljana, Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Novo mesto, Trška gora, WL17, 11. 6. 1992, V. Furlan leg.

Goričko: Sotina, r. Ledava, WM78, 30. 7. 1998, S. Brelih leg.

Prekmurje: Bukovnica, XM07, 2. 6. 1999, S. Brelih leg. Nova Gorica, Panovec, UL98, 21. 4. 2000, 15. 5. 2000, S. Brelih leg.

Ratitovec, 1100 m, VM22, 6. 6. 1982, V. Furlan leg. Podkum, 740 m, WM00, 24. 5. 1989, V. Furlan leg. Goričko: Dolič, WM88, 25. 5. 1989, S. Brelih leg. Ljubljana: Dobrunje, Sv. Urh, VL69, 25. 6. 1997, V. Furlan leg.

Novo Mesto, Šmarjeta, WL18, 3. 5. 1997, V. Furlan leg.

#### Cymus claviculus (Fallén, 1807)

Montandon, 1886: Gorica; Gogala & Gogala, 1986; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Podčetrtek, WM41, 10. 5. 1933, E. Jaeger leg. Pokljuka, Planina Lipanca, VM13, 2. 9. 1979, A. & M. Gogala leg.

Topol, Sv. Katarina, VM50, 2. 6. 1991, V. Furlan leg.

#### Cymus glandicolor Hahn, 1832

Gogala & Moder, 1960: Ljubljana, Dobrova, Bled, Lancovo; Gogala & Gogala, 1986, 1989, 1994; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Veldes (= Bled), VM33, 19. 7. 1931, Staudacher leg. Radovljica, Lancovo, VM33, 28. 7. 1929, Staudacher leg.

Ljubljansko barje: Plešivica, VL59, 23. 4. 1978, A. & M. Gogala leg.

Log, Lukovica, VL59, 2. 6. 1978, A. & M. Gogala leg. Črni kal, VL14, 15. 4. 1979, A. & M. Gogala leg. Ljubljana, Škofljica, VL69, 19. 5. 1979, A. & M. Gogala leg.

Ljubljana: Šiška, VM50, 1. 5. 1980, A. Gogala leg. Velike Lašče, VL77, 25. 5. 1980, A. & M. Gogala leg. lg, Kremenica, VL68, 30. 4. 1981, S. Brelih leg. Kamniško-Savinjske Alpe: Krvavec, VM62, 14. 6. 1981, A. & M. Gogala leg.

Istra: Koštabona, VL03, 25. 6. 1981, M. Gogala leg. Cerknica, Begunje, Topol, VL57, 28. 6. 1981, A. & M. Gogala leg.

Ig, Iška loka, VL69, 10. 7. 1982, A. & M. Gogala leg. Prekmurje: Dobrovnik, Bukovniško jezero, XM07, 30. 4. 1983, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 7. 8. 1983, 19. 4. 1987, A. & M. Gogala leg.

Bloke: Volčje, VL67, 12. 5. 2001, A. Gogala leg.

Osilnica, Plešce, Slovene side of the river, VL74, 27. 7. 1985, A. & M. Gogala leg.

Rakitna, VL58, 22. 6. 1986, A. & M. Gogala leg. Movraž, Movraška vala, VL13, 18. 5. 1990, A. & M. Gogala leg.

Bizeljsko, Stara vas, WL59, 21. 4. 2000, A. Gogala leg. Kras: Povir, Brestovica, Studence, VL16, 14. 5. 2000, A. Gogala leg.

Planina, Planinsko polje, VL47, 21. 6. 2000, A. Gogala leg.

Pokljuka: Barje Šijec, VM23, 5. 8. 2003, A. & M. Gogala leg.

Pokljuka: Mrzli Studenec, barje ob cesti, VM23, 5. 8. 2003, A. & M. Gogala leg.

Gotenica, 600 m, VL85, 4. 7. 1997, S. Brelih leg. Ljubljana, Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Podkum, Medvedov graben, WM00, 16. 4. 1991, V. Furlan leg.

Ljubljana, Lavrica, VL69, 29. 4. 1991, V. Furlan leg. Loški potok, VL66, 31. 7. 1997, V. Furlan leg. Loški potok: Retje, VL66, 14. 5. 1990, V. Furlan leg. Kozje, Podsreda, Trebča Gorca, WM40, 9. 7. 1998, S. Brelih leg.

Prekmurje: Bukovnica, XM07, 2. 6. 1999, S. Brelih leg. Sočerga, Šeki, VL13, 14. 6. 1999, 11. 5. 2000, S. Brelih leg.

Dragonja, UL93, 4. 5. 2000, S. Brelih leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, 6. 7. 2000, S. Brelih leg.

Ljubljana, Barje, VL69, 1. 5. 1980, 13. 5. 1982, V. Furlan leg.

Muljava, VL88, 27. 5. 1982, V. Furlan leg. Ratitovec, 1100 m, VM22, 6. 6. 1982, V. Furlan leg. Gorjanci: Jugorje, WL16, 27. 4. 1983, V. Furlan leg.

Suha krajina: Ambrus, VL87, 7. 5. 1983, V. Furlan leg. Suha krajina: Struge: Lipa, VL87, 7. 5. 1983, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 2. 6. 1984, V. Furlan leg.

Podkum, 740 m, WM00, 24. 5. 1989, V. Furlan leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

Črni Kal, Praproče, VL14, 12. 7. 1990, V. Furlan leg. Krka, VL88, 7. 6. 1987, S. Brelih leg.

# Cymus melanocephalus Fieber, 1861

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana, Dobrova; Gogala & Gogala, 1986, 1989, 1994; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Laibach (= Ljubljana), 2. 9. 1931, 31. 5. 1933, Staudacher leg.

Koper, Škocjanski zatok, VL04, 1. 7. 1979, A. & M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 14. 5. 2000, A. Gogala leg.

Ljubljansko barje: Bevke, VL59, 14. 6. 1980, A. & M. Gogala leg.

Ljubljana: Šiška, VM50, 3. 9. 1980, A. & M. Gogala leg. Istra: Koštabona, VL03, 25. 6. 1981, M. Gogala leg., 7. 8. 1986, A. & M. Gogala leg.

Podgorski kras: Petrinje, VL14, 8. 6. 1983, A. & M. Gogala leg.

Osilnica, Plešce, Slovene side of the river, VL74, 27. 7. 1985, A. & M. Gogala leg.

Horjul, Lesno brdo, VL49, 8. 6. 1986, A. & M. Gogala leg.

Prekmurje: Gomilica, XM06, 13. 6. 1987, A. & M. Gogala leg.

Petišovci, XM15, 13. 6. 1987, A. & M. Gogala leg.

Sp. Brnik, VM61, 2. 9. 1988, M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Movraž, Movraška vala, VL13, 18. 5. 1990, A. & M. Gogala leg.

Vinje pri Moravčah, VM71, 23. 5. 1997, A. Gogala leg. Bizeljsko, Stara vas, WL59, 21. 4. 2000, A. Gogala leg. Povir, Brestovica, Studence, VL16, 14. 5. 2000, A. Gogala leg.

Moravče, Prikrnica, ob Drtijščici, VM71, 19. 5. 1997, S. Brelih leg.

Novo mesto, Trška gora, WL17, 21.-22. 5. 1983, 11. 6. 1992, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 21. 5. 1991, V. Furlan leg.

Nova Gorica, Panovec, UL98, 21. 4. 2000, S. Brelih leg. Kranj, Brdo, VM52, 14. 7. 2006, A. Gogala leg. Ljubljana: Šmartno, VM60, 4. 5. 1988, V. Furlan leg.

Prekmurje: Andrejci, WM97, 23. 5. 1989, S. Brelih leg.

# Blissinae

# Dimorphopterus blissoides (Baerensprung, 1859)

Gogala & Gogala, 1986, 1989; A. Gogala, 1992; Péricart, 1998a: Sečovlje

Specimens examined:

Istra: Sečovlje, Fontanigge, UL93, 21. 9. 1982, 30. 8. 1989, A. & M. Gogala leg., 2. 10. 1986, M. Gogala leg., 6. 5. 2000, 17. 9. 2002, 16. 9. 2005 on *Phragmites*, A. Gogala leg.

# Dimorphopterus spinolae (Signoret, 1857)

Gogala & Moder, 1960: Jezersko; Protić, 1987: Podčetrtek, E. Jaeger leg.; A. Gogala, 1996; Péricart, 1998a: Ljubljana

Specimens examined:

Novo mesto, Trška gora, WL17, 4. 5. 1997, V. Furlan leg.

Radeče, Jagnjenica, WM00, 24. 5. 1990, V. Furlan leg.

#### Ischnodemus quadratus Fieber, 1837

A. Gogala, 1991; Gogala & Gogala, 1994

Specimens examined:

Strunjan, UL94, 30. 9. 1979, 17. 9. 1989, A. & M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 18. 5. 1980, A. & M. Gogala leg., 6. 5. 2000, A. Gogala leg., 23. 5. 2000, S. Brelih leg.

Marezige, VL04, 7. 9. 1985, A. Gogala leg.

Istra: Boršt, VL03, 3. 5. 1986, A. & M. Gogala leg.

Sečovlje, Fontanigge, UL93, 21. 9. 1982, A. & M. Gogala leg., 6. 5. 2000, A. Gogala leg.

Movraž, Movraška vala, VL13, 18. 5. 1990, A. & M. Gogala leg.

Vipavska dolina: Renče, UL98, 22. 7. 1980, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

Braniška dolina: Kodreti, Dolanci, VL17, 2. 5. 2000, A. & M. Gogala leg.

Istra: Kozloviči, VL03, 9. 7. 1997, S. Brelih leg.

Bled, Koritno, VM33, 23. 7. 1996, B. Drovenik leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, 13. 9. 2000, 10. 5. 2001, S. Brelih leg.

Sočerga, Šeki, VL13, 11. 5. 2000, S. Brelih leg. Ankaran, Valdoltra, VL04, 23. 4. 2002, S. Brelih leg.

#### Ischnodemus sabuleti (Fallén, 1826)

Gogala & Moder, 1960: Lendava; Gogala & Gogala, 1986, 1989: records refer to *I. quadratus* Specimens examined:

Prekmurje: Lendava, XM15, Staudacher leg.

Pomurje: Rihtarovci, WM86, 13. 6. 1996, B. Drovenik leg., 10. 4. 1997 on *Phragmites*, S. Brelih leg.

Maribor, Rače, Veliki ribnik, WM54, 12. 5. 1992, V. Furlan leg.

Karavanke: Pl. Pungrat, 1440 m, VM54, 20. 6. 2000, S. Brelih leg.

Petišovci, Muriša, XM15, 23. 5. 1996, S. Gomboc & D. Kofol leg.

#### Henestarinae

# Henestaris halophilus (Burmeister, 1835)

? Henestaris geocoriceps D'Antessanty, 1885

Montandon, 1886: Gorica; Gogala & Moder, 1960: Strunjan; Gogala & Gogala, 1986; A. Gogala, 1992 Specimens examined:

Istra: Strunjan, UL94, 30. 9. 1979, A. & M. Gogala leg. Sečovlje, Fontanigge, UL93, 21. 9. 1982, A. & M. Gogala leg., 12. 8. 1997, 6. 5. 2000, A. Gogala leg. Koper, Bertoki, Škocjanski zatok, VL04, 14. 5. 2000, A. Gogala leg.

#### Geocorinae

#### Geocoris ater (Fabricius, 1787)

A. Gogala, 1991

Specimens examined:

Prekmurje: Lendava, XM15, Staudacher leg.

Brestovica pri Komnu, Drenovce, UL97, 28. 6. 2005, S.

Brelih leg.

#### *Geocoris megacephalus* (Rossi, 1790)

Geocoris siculus (Fieber, 1844)

Gogala & Moder, 1960: Strunjan; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Istra: Portorož, Lucija, UL94, 2. 7. 1983, A. & M. Gogala leg.

Pororož, Beli križ, UL84, 10. 10. 1984, M. Gogala leg. Portorož, UL94, 15. 10. 1986, A. & M. Gogala leg. Kraški rob: Črni kal, VL14, 1. 11. 1986, A. & M. Gogala leg. leg.

Sečovlje, ob Dragonji, UL93, 7. 6. 1984, S. Brelih leg. Dragonja, Stena, UL93, 1. 10. 1987, A. & M. Gogala leg.

Ankaran, VL04, 17. 2. 1988, A. & M. Gogala leg. Izvir Rižane, VL14, 18. 2. 1990, A. & M. Gogala leg. Braniška dolina: Kodreti, Dolanci, VL17, 2. 5. 2000, A. & M. Gogala leg.

Strunjan, Belvedere, UL94, 5. 10. 2000, A. Gogala leg. Sočerga, Gradec, VL13, 28. 10. 2000, A. Gogala leg. Strunjan, rt Ronek, UL94, 3. 9. 1998, 6. 9. 1998, V. Furlan leg.

# Geocoris erythrocephalus (Lepeletier & Serville, 1825) Montandon, 1886: Gorica; Gogala & Gogala, 1989, 1994

Specimens examined:

Istra: Labor, dolina Dragonje, VL03, 9. 9. 1987, A. Gogala leg.

Pomjan, VL03, 9. 6. 1990, A. & M. Gogala leg. Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Bezovica, VL14, 14. 6. 1991, A. & M. Gogala leg. Kraški rob: Podpeč, VL14, 24. 8. 1991, 13. 6. 1992, A. & M. Gogala leg.

Ankaran, VL04, 20. 7. 1997, B. Drovenik leg. Gračišče, Butari, VL13, 19. 9. 1998, A. Gogala leg. Koper, Bertoki, Škocjanski zatok, VL04, 22. 7. 2000, A. Gogala leg., 23. 5. 2000, 23. 4. 2002, S. Brelih leg. Istra: Zanigrad, VL14, 7. 7. 2003, S. Brelih leg. Strunjan, rt Ronek, UL94, 3. 9. 1998, 4. 9. 1998, 6. 9. 1998, V. Furlan leg.

#### **Artheneinae**

*Chilacis typhae* (Perris, 1857) Gogala & Gogala, 1986, 1989 Specimens examined:

Ig, Draga, VL68, 29. 11. 1983 on *Typha*, A. & M. Gogala leg.

Log, Lukovica, VL59, 13. 2. 1986, M. Gogala leg. Bevke, breg Ljubljanice, VL59, 28. 3. 1999 on *Typha*, A. Gogala leg.

Bevke, Mali plac, VL59, 3. 2001 ex larva on *Typha*, A. Gogala leg.

Ljubljansko barje: Ig, VL69, 25. 6. 1997, V. Furlan leg. Ljubljana, VL69, 16. 11. 1986, V. Furlan leg. Ljubljana, VM60, 29. 3. 1987, V. Furlan leg.

#### Heterogastrinae

#### Heterogaster affinis Herrich-Schaeffer, 1835

Gogala & Moder, 1960: Preserje, Šmartno ob Savi; A. Gogala, 1996

Specimen examined:

Kras: Komen, Kregolišče, VL07, 16. 6. 1992, A. Gogala leg.

# Heterogaster artemisiae Schilling, 1829

Gräffe, 1911: Tolmin; Gogala & Gogala, 1986, 1989 Specimens examined:

Ljubljana: Ježica, VM60, 25. 3. 1934, Staudacher leg. Istra: Padna, UL93, 16. 6. 1984, A. & M. Gogala leg. Polhograjsko hrib.: Črni vrh, Pasja ravan, VM40, 15. 8. 1984, 4. 6. 1985, A. & M. Gogala leg.

Brje pri Komnu, VL07, 7. 9. 1989, A. & M. Gogala leg. Senožeče, Gabrška gora, VL26, 18. 4. 1992, A. & M. Gogala leg.

Zaplana, Strmica, VL49, 11. 1. 1998, M. Gogala leg. Kras: Trstelj, UL98, 25. 6. 2000, A. & M. Gogala leg. Sežana, Štorje, VL16, 16. 5. 1984, V. Furlan leg.

#### Heterogaster urticae (Fabricius, 1775)

Gogala & Moder, 1960: Ljubljana, Dobrova; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Ig, Kremenica, VL68, 16. 9. 1975, 21. 9. 1975, S. Brelih leg.

Vrhnika, Log, VL59, 7. 3. 1981, A. & M. Gogala leg. Log, Lukovica, VL59, 4. 2. 1983, 16. 8. 1983, 7. 2. 1988, A. & M. Gogala leg.

Ljubljana: Ilovica, VL69, 10. 9. 1983, A. & M. Gogala leg.

Ljubljansko barje: Bevke, VL59, 2. 2. 1985, A. & M. Gogala leg.

Vrhnika, Drenov grič, VL49, 18. 2. 1989, M. Gogala leg. Slavnik, VL14, 9. 7. 1995, A. & M. Gogala leg. Kozina, VL15, 22. 6. 1991, V. Furlan leg. Brezje pri Dobrovi, VL49, 20. 8. 1986, M. Zdešar leg. Ljubljana, Ljubljansko barje, VL69, 11. 8. 1983, V. Fur-

lan leg.

# Platyplax salviae (Schilling, 1829)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana: Ježica, Šklendrovec, Trenta: pod Javorščkom, Črni kal; Gogala & Gogala, 1986, 1989, 1994; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Šklendrovec, WM00, 12. 6. 1932, Staudacher leg. Ljubljana: Ježica, VM60, 26. 5. 1938, Staudacher leg. Ljubljana, Škofljica, VL69, 19. 5. 1979, A. & M. Gogala leg.

Črni kal, VL14, 28. 6. 1980, A. & M. Gogala leg. Kras: Štorje, VL16, 22. 7. 1980, A. & M. Gogala leg., 16. 5. 1984, V. Furlan leg.

Istra: Koštabona, VL03, 25. 6. 1981, M. Gogala leg., 7. 6. 1987, A. & M. Gogala leg.

Brkini: Barka, VL25, 28. 7. 1984, A. & M. Gogala leg. Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

Brje pri Komnu, VL07, 5. 5. 1989, A. & M. Gogala leg. Brestovica pri Komnu, UL97, 2. 5. 1990, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 18. 5. 1990, A. & M. Gogala leg., 16. 5. 1990, V. Furlan leg.

Hrastnik, WM01, 8. 5. 1998, M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 25. 8. 2005 on *Calluna vulgaris*, A. Gogala leg.

Popetre, 300 m, VL03, 9. 7. 1997, S. Brelih leg.

Hrpelje, Prešnica, VL14, 6. 7. 1998, 10. 5. 1999, S. Brelih leg.

Črnotiče, 390 m, VL14, 1. 5. 2002, S. Brelih leg. Lipica, VL15, 19. 5. 1979, 30. 5. 1982, 25. 5. 1985, V. Furlan leg.

Divača, VL16, 30. 5. 1982, V. Furlan leg.

Vremščica, VL26, 11. 5. 1981, 10. 5. 1987, V. Furlan leg.

Senožeče, Gabrče, VL26, 20. 6. 1982, 26. 5. 1987, V. Furlan leg.

Ljubljana, Ljubljansko barje, VL69, 13. 5. 1982, V. Furlan leg.

Gornji Ig, 600 m, VL68, 5. 6. 1982, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 4. 6. 1983, V. Furlan leg

Novo mesto, Trška gora, WL17, 21. – 22. 5. 1983, 6. 6. 1987, 4. 5. 1997, V. Furlan leg.

Povir, VL16, 31. 7. 1984, V. Furlan leg.

Krka, VL88, 7. 6. 1987, S. Brelih leg.

Kočevska Reka, Borovška dolina, VL84, 15. 6. 1993, V. Furlan leg.

Mrzlica, 1100 m, WM01, 13. 6. 1991, V. Furlan leg. Ljubljana, Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Postojna, VL37, 10. 6. 1991, V. Furlan leg. Črni kal, Socerb, VL15, 8. 5. 1990, V. Furlan leg. Črni kal, Črnotiče, VL14, 8. 5. 1990, V. Furlan leg. Brežec pri Podgorju, VL14, 16. 5. 1990, V. Furlan leg. Zasavje: Podkum, WM00, 24. 5. 1989, V. Furlan leg. Loški potok, VL66, 8. 6. 1997, 21. 6. 1997, V. Furlan leg.

Terme Čatež, WL48, 25. 4. 1998, V. Furlan leg. Trnovski gozd: Lokve, VL09, 27. 6. 1998, V. Furlan leg.

#### Oxycareninae

Brachyplax tenuis (Mulsant & Rey, 1852)

Brachyplax palliata (A. Costa, 1853)

Gogala & Gogala, 1989

Specimens examined:

İstra: Portorož, UL94, 1. 10. 1987, A. & M. Gogala leg.

#### Macroplax fasciata (Herrich-Schaeffer, 1835)

Oxycarenus helferi Fieber, 1837

Montandon, 1886: Gorica; Gogala & Moder, 1960: Črni kal; A. Gogala, 1991; Gogala & Gogala, 1994 Specimen examined:

Istra: Osp, VL14, 8. 7. 1990, A. & M. Gogala leg. Sočerga, Šeki, VL13, 17. 6. 1999, S. Brelih leg.

# *Macroplax preyssleri* (Fieber, 1837)

Gogala & Gogala, 1986, 1989, 1994; Péricart, 1998b: Ljubljana

Specimens examined:

Slavnik, VL14, 2. 7. 1982 on *Helianthemum*, A. & M. Gogala leg., 23. 6. 1991, V. Furlan leg.

Kras: Sežana, Povir, VL16, 8. 6. 1983, A. & M. Gogala leg.

Istra: Padna, UL93, 16. 6. 1984, A. & M. Gogala leg. Sorica, Soriška planina, VM22, 23. 6. 1984, A. & M. Gogala leg.

Sočerga, VL13, 26. 7. 1984, A. & M. Gogala leg. Brkini: Barka, VL25, 28. 7. 1984, A. & M. Gogala leg. Polhograjsko hrib.: Črni vrh, Pasja ravan, VM40, 15. 8. 1984, A. & M. Gogala leg.

Koštabona, VL03, 7. 6. 1987, A. & M. Gogala leg. Uršlja gora: Plešivec, VM94, 22. 8. 1987, A. & M. Gogala leg.

Labor, VL03, 9. 9. 1987, A. Gogala leg.

Brje pri Komnu, VL07, 12. 6. 1989, M. Gogala leg., 7. 9. 1989, 10. 10. 1999, A. & M. Gogala leg.

Vremščica, VL26, 4. 7. 1992, A. & M. Gogala leg.

Vremščica, 800 m, VL26, 11. 6. 2000, A. & M. Gogala leg.

lg, Škrilje, Stražar, 720 m, VL68, 14. 6. 1999, A. & M. Gogala leg.

Zazid, Zalipnik, VL13, 26. 5. 2000, A. Gogala leg. Polhograjsko hrib.: Grmada, 898 m, VM40, 11. 6. 2000, A. Gogala leg.

Sočerga, Šeki, VL13, 14. 6. 1999, S. Brelih leg.

Hrpelje, Prešnica, VL14, 23. 5. 1999, 7. 6. 1999, S. Brelih leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg. Črni kal, Praproče, VL14, 22. 5. 1990, V. Furlan leg. Brežec pri Podgorju, VL14, 16. 5. 1990, V. Furlan leg.

Sočerga, Mlini, Veli Badin, VL13, 12. 6. 1990, V. Furlan leg.

Loški potok: Retje, VL66, 14. 5. 1990, 30. 7. 1998, V. Furlan leg.

Loški potok, VL66, 21. 6. 1997, V. Furlan leg. Kozina, VL15, 22. 6. 1991, V. Furlan leg. Kozina, Prešnica, VL14, 22. 6. 1991, V. Furlan leg. Mrzlica, 1100 m, WM01, 25. 6. 1991, V. Furlan leg. Topol, Sv. Katarina, VM50, 9. 6. 1991, V. Furlan leg. Muljava, VL88, 12. 8. 1998, V. Furlan leg. Mozirje, Šmihel, VM93, 15. 8. 1998, V. Furlan leg.

# Metopoplax origani (Kolenati, 1845)

Gogala & Gogala, 1986

Specimens examined:

Podčetrtek, WM41, 31. 5. 1933, E. Jaeger leg. Kras: Brje pri Komnu, VL07, 29. 6. 2003, A. & M. Gogala leg.

# Microplax albofasciata (A. Costa, 1847)

Gogala & Moder, 1960: Bela krajina: Črnomelj, Tribuče (WL14)

#### Microplax interrupta (Fieber, 1837)

Péricart, 2001a

Specimen examined:

Kras: Komen, Vale, UL97, 9. 4. 2000, A. & M. Gogala leg.

# Oxycarenus pallens (Herrich-Schaeffer, 1850)

Gogala & Moder, 1960: Črni kal; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Koper, Škocjanski zatok, VL04, 1. 7. 1979, A. & M. Gogala leg.

Ankaran, VL04, 8. 6. 1983, A. & M. Gogala leg. Portorož, Lucija, UL94, 2. 7. 1983, A. & M. Gogala leg. Marezige, VL04, 7. 9. 1985, A. Gogala leg. Istra: Pomjan, VL03, 7. 9. 1985, A. Gogala leg.

Uršlja gora: Poštarska koča, VM94, 22. 8. 1987, A. & M. Gogala leg.

Sežana, Štorje, VL16, 16. 5. 1984, V. Furlan leg. Kras: Brje pri Komnu, VL07, 21. 7. 1990, A. & M. Gogala leg.

Sečovlje, Fontanigge, UL93, 24. 8. 1991, 12. 9. 1992, A. & M. Gogala leg., 18. 9. 2003, A. Gogala leg. Zazid, Zalipnik, VL13, 26. 5. 2000, A. Gogala leg. Zazid, Lipnik, 800 m, VL13, 17. 6. 2000, A. Gogala leg. Strunjan, rt Ronek, UL94, 3. 9. 1998, V. Furlan leg.

#### Oxycarenus lavaterae (Fabricius, 1787)

Montandon, 1886: Gorica; Gogala & Gogala, 1988, 1989

Specimens examined:

Nova Gorica, UL99, 11. 11. 1987 on *Tilia*, G. Seljak leg. Istra: Strunjan, UL94, 12. 9. 1992, A. & M. Gogala leg.

Ljubljana, VM60, 7. 8. 1995, M. Gogala leg. Ljubljana: Vič, VM50, 23. 10. 2000, L. Šercelj leg. Ljubljana: Vič, Postojnska ul., VM60, 21. 1. 2003, M. Lozar Štamcar leg. Ljubljansko barje: Log pri Brezovici, VL59, 19. 7. 2004, M. Gogala leg.

#### Oxycarenus modestus (Fallén, 1829)

Oxycarenus spitzyi Fieber, 1837

Fieber, 1837, 1851: Krain (= Carniola) – type locality of *O. spitzyi*; Montandon, 1886: Gorica; Gogala & Gogala, 1986, 1989

Specimens examined:

Prekmurje: Moravci, WM97, 30. 4. 1983 under *Alnus*, A. & M. Gogala leg.

Ljubljansko barje: Brezovica, VL59, 1. 5. 1984 on *Alnus*, 27. 4. 1986, A. & M. Gogala leg.

Dobrovnik, Bukovniško jezero, XM07, 23. 5. 1992, A. & M. Gogala leg.

Ljubljana: Zadvor, VL69, 30. 4. 1985, V. Furlan leg.

# Rhyparochrominae

Antillocorini

#### Tropistethus holosericeus (Scholtz, 1846)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Dolnice, Tomačevo; Gogala & Gogala, 1986, 1989 Specimens examined:

Ljubljana: Tomačevo, VM60, 25. 4. 1944, Staudacher leg.

Ljubljana: Dolnice, VM50, 10. 4. 1944, Staudacher leg. Istra: Izola, UL94, 22. 9. 1982, A. Gogala leg. Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala

leg. Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg.

Kras: Brestovica pri Komnu, UL97, 2. 5. 1990, A. & M. Gogala leg.

Komen, Vale, UL97, 9. 4. 2000, A. & M. Gogala leg. Brje pri Komnu, VL07, 22. 5. 2005, A. Gogala leg. Ljubljana: Rakovnik, VL69, 9. 4. 1991, V. Furlan leg. Sočerga, Šeki, VL13, 11. 5. 2000, S. Brelih leg. Gorenje pri Divači, VL16, 26. 5. 2004, S. Brelih leg. Koper, Škocjanski zatok, VL04, 23. 4. 2002, S. Brelih leg.

# Drymini

#### Drymus latus Douglas & Scott, 1871

Drymus confusus Horváth, 1881

Montandon, 1886: Gorica; Gogala & Gogala, 1986; Péricart, 1998b: Gorenjska Specimens examined:

Ljubljana: Ježica, VM60, 3. 9. 1933, Staudacher leg. Grosuplje, Velike Lipljene, VL78, 30. 8. 1981, A. & M. Gogala leg.

Grosuplje, Polica, VL79, 24. 8. 1982, A. & M. Gogala leg.

#### Drymus pilicornis (Mulsant & Rey, 1852)

Gogala & Moder, 1960: Golica (VM24)

Specimen examined:

Banjaloka, Stružnica, VL83, 28. 4. 2004, S. Brelih leg.

#### Drymus pilipes Fieber, 1861

Montandon, 1886: Gorica; Protić, 1987: Podčetrtek, E. Jaeger leg.

#### Drymus brunneus (R.F. Sahlberg, 1848)

Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 1. 11. 1944, Staudacher leg. Ljubljansko barje: Brezovica, VL59, 9. 4. 1983, A. & M. Gogala leg.

Gornja Radgona, Okoslavci, WM76, 23. 4. 1980

Prekmurje: Murski Petrovci, WM86, 11. 4. 1997, S. Brelih leg.

Ljubljana, Ljubljansko barje, VL69, 26. 2. 1992, V. Furlan leg.

Slovenske gorice: Drbetinci, WM75, 23. 4. 1998, S. Brelih leg.

Breginj, Podbelo, UM73, 6. 1982, B. Drovenik leg.

#### Drymus ryeii Douglas & Scott, 1865

Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 20. 10. 1928, Staudacher leg. Ljubljansko barje: Plešivica, VL59, 23. 4. 1978, A. & M. Gogala leg.

Bohinj: Ukanc, VM02, 24. 4. 1979, A. & M. Gogala leg. Škofja Loka, VM41, 13. 5. 1979, A. & M. Gogala leg. Vrhnika, Log, VL59, 7. 3. 1981, A. & M. Gogala leg. Bela krajina: Preloka, WL23, 13. 9. 1981, A. & M. Gogala leg.

Bevke, VL59, 27. 3. 1982, A. & M. Gogala leg. Log, Lukovica, VL59, 20. 3. 1983, A. & M. Gogala leg. Ljubljana: Savlje, VM60, 19. 4. 1984, A. & M. Gogala leg.

Kras: Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg. Sorica, Soriška planina, VM22, 23. 6. 1984, A. & M. Gogala leg.

Velike Bloke, VL57, 19. 4. 1987, A. & M. Gogala leg. Žužemberk, VL97, 5. 3. 1989, A. & M. Gogala leg. Ljubljana, Beričevo, VM70, 9. 11. 1996, A. & M. Gogala leg

Polhograjsko hrib.: Topol, Sv. Katarina, VM50, 7. 2. 1998, A. & M. Gogala leg.

Škocjan, Naklo, desni breg Reke, VL25, 11. 10. 2001, A. Pirnat leg.

Vrhe: Stomaž, p. Kranjšek, VL17, 28. 7. 2005, A. Gogala leg.

Ljubljana, Golovec: Orle, VL69, 4. 5. 1984, 21. 4. 1985, 1. 4. 1991, 13. 5. 1991, V. Furlan leg.

Ilirska Bistrica, Zarečje – Brce, VL34, 31. 5. 1999, S. Brelih leg.

Dolina Kolpe: Sp. Bilpa – Vrt, 200 m, VL93, 29. 4. 2001, S. Brelih leg.

Ribnica, Ugar, the Ribnica river, VL76, 16. 2. 2001, S. Brelih leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg.

Kurešček, VL68, 5. 6. 1983, V. Furlan leg.

Letuš, Dobrovlje, VM92, 6. 1984, B. Drovenik leg. Topol, Robež, VM50, 28. 4. 1990, V. Furlan leg.

#### **Drymus sylvaticus** (Fabricius, 1775)

Gogala & Moder, 1960: Ljubljana, Golica; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Ljubljana: Savlje, VM60, 5. 3. 1977, 13. 3. 1983, 21. 4. 1984, A. & M. Gogala leg.

Radovljica, VM33, 4. 3. 1979, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 8. 4. 1979, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 10. 8. 1983, A. & M. Gogala leg.

Slavnik, VL14, 2. 6. 1984, 23. 6. 1991, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Ljubljana, Ljubljansko barje, VL69, 26. 2. 1992, V. Furlan leg.

Podkum, 740 m, WM00, 24. 5. 1989, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. Loški potok, VL66, 17. 5. 1997, V. Furlan leg. Krma, VM14, 14. 5. 1983, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 23. 4. 1983, 4. 5. 1984, V. Furlan leg.

## Eremocoris abietis (Linnaeus, 1758)

Gogala & Moder, 1960: Portorož

#### Eremocoris fenestratus (Herrich-Schaeffer, 1839)

Montandon, 1886: Gorica; Protić, 1987: Savinjske planine, E. Jaeger leg.; A. Gogala, 1991: Brje pri Komnu, Dragonja; Péricart, 1998b: Postojna

Specimens examined:

Kras: Brje pri Komnu, VL07, 25. 2. 1990, A. & M. Gogala leg., 1. 3. 1997 on *Pinus nigra*, A. Gogala leg., 4. 4. 2004, M. Gogala leg.

Komen, Vale, UL97, 10. 10. 1999, A. & M. Gogala leg.

### Eremocoris plebejus (Fallén, 1807)

Gogala & Moder, 1960: Ljubljana, Pokojišče; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 12. 4. 1931, Staudacher leg. Pokojišče, VL58, 19. 5. 1929, 14. 5. 1931, Staudacher leg.

Medvode, Preska, VM50, 4. 7. 1981, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, A. & M. Gogala leg.

Kočevje, Strmec virgin forest, VL85, 25. 6. 1994, A. & M. Gogala leg.

Goričko: Kančevci, WM98, 26. 5. 1997, S. Brelih leg. Ljubljana, Golovec: Orle, VL69, 27. 3. 1989, 13. 5. 1991, V. Furlan leg.

Ljubljana, Golovec, VL69, 30. 5. 1976, 21. 5. 1989, V. Furlan leg.

Ajdovščina, Podčaven, VL18, 6. 1981, B. Drovenik leg. Polhograjsko hrib.: Grmada, 800 m, VM40, 19. 3. 1983, V. Furlan leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg.

Podolševa, Kisla voda, VM74, 7. 1986, B. Drovenik leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. Podkum, Sopota, WM00, 28. 3. 1989, V. Furlan leg. Ljubljana, Črnuče, VM60, 6. 4. 1987, V. Furlan leg. Loški potok: Retje, VL66, 14. 5. 1990, V. Furlan leg.

#### Eremocoris podagricus (Fabricius, 1775)

Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Kras: Komen, Mali Dol, VL07, 9. 1981, B. Drovenik leg. Cerkniško jezero: Dolenje Jezero, VL56, 24. 5. 1987, A. & M. Gogala leg.

Kras: Veliki Dol, VL07, 22. 3. 1992, A. & M. Gogala leg. Vipavska dolina: Ozeljan, Lijak, UL99, 8. 4. 1992, S. Brelih leg.

Kraški rob: Zazid, VL13, 23. 10. 2000, A. Gogala leg. Osp, Osapska jama, 100 m, VL14, 26. 9. 2001, A. Pirnat leg.

Škocjanske jame: Mala dolina, VL25, 29. 4. 2004, A. Gogala leg.

Sp. Šklendrovec, WM00, 16. 4. 1991, V. Furlan leg. Dolina Kolpe: Sp. Bilpa – Vrt, 200 m, VL93, 29. 4. 2001, S. Brelih leg.

Tolmin, Podljubinj, VM01, 8. 1982, B. Drovenik leg. Ljubljana, Golovec: Orle, VL69, 4. 5. 1984, V. Furlan leg.

Bela krajina: Preloka, WL23, 3. 5. 1989, B. Drovenik leg.

Kum, WM00, 9. 7. 1987, B. Drovenik leg.

#### Gastrodes abietum Bergroth, 1914

? Cimex abietis Scopoli, 1763 (non Linnaeus, 1758) Gogala & Moder, 1960: Ljubljana, Vižmarje; Gogala & Gogala, 1986

Specimens examined:

Ljubljana: Vižmarje, VM50, 16. 11. 1930, Staudacher leg.

Julijske Alpe: Vrata, VM14, 5. 7. 1949, E. Pretner leg. Ljubljana: Šiška, VM50, 13. 1. 1980, 8. 2. 1980, A. & M. Gogala leg.

Moravče, Limbarska gora, VM81, 10. 2. 1980, A. & M. Gogala leg.

Pokojišče, VL48, 7. 9. 1981, A. & M. Gogala leg.

Selška dolina: Dolenja vas, VM41, 5. 3. 1982 in cones Ljubljansko barje: Log, Lukovica, VL59, 5. 3. 1983, 20. 3. 2006, A. & M. Gogala leg.

Preserje, Sv. Ana, VL59, 20. 2. 1999, A. Gogala leg. Polhograjsko hrib.: Setnica, Grmada, 860 m, VM40, 17. 10. 2000, A. Gogala leg.

Istra: Strunjan, Belvedere, UL94, 24. 3. 2001, A. Gogala leg.

Topol, Sv. Katarina, VM50, 20. 3. 1982, V. Furlan leg. Ljubljana, VM60, 8. 1. 1983, V. Furlan leg.

Gorjanci: sedlo, 635 m, WL16, 27. 4. 1983, V. Furlan leg.

# Gastrodes grossipes (De Geer, 1773)

Gogala & Moder, 1960: Ljubljana, Vižmarje, Pokojišče; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Pokojišče, VL58, 7. 7. 1932, Staudacher leg., VL48, 19. 8. 1984 on *Pinus*, A. & M. Gogala leg.

Ljubljana: Vižmarje, VM50, 16. 11. 1930, Staudacher leg.

Ljubljana: Rožnik, VM50, 25. 1. 1954 under *Picea abies* bark, M. Gogala leg.

Bohinj: Stara Fužina, VM12, 3. 2. 1979 under *Picea* bark, A. & M. Gogala leg.

Ljubljana: Šiška, VM50, 13. 1. 1980, A. & M. Gogala leg.

Moravče, Limbarska gora, VM81, 10. 2. 1980, A. & M. Gogala leg.

Kamnik pod Krimom, 2 km S, VL58, 4. 10. 1981 on *Juniperus*, A. & M. Gogala leg.

Velike Bloke, Ulaka, VL57, 7. 8. 1983, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 7. 8. 1983, 11. 7. 1987, A. & M. Gogala leg.

Kras: Štanjel, Kopriva, VL07, 2. 8. 1985 on *Pinus*, A. & M. Gogala leg.

Ljubljansko barje: Log, Lukovica, VL59, 29. 11. 1989, A. & M. Gogala leg.

Istra: Sočerga, Veli Badin, VL13, 4. 2. 1990, 3. 10. 1990, A. & M. Gogala leg.

Brje pri Komnu, VL07, 1. 3. 1997 on *Pinus nigra*, A. Gogala leg.

Škofja Loka, Lubnik, Suša, VM41, 17. 1. 1999, M. Štangelj leg.

Istra: Zazid, 380 m, VL14, 26. 5. 2003, S. Brelih leg. Kozina, Petrinje, VL14, 30. 4. 1980, M. Zdešar leg. Ljubljana, Golovec, VL69, 26. 4. 1980, V. Furlan leg. Ljubljana, VM60, 3. 3. 1982, V. Furlan leg. Corkniško jozoro: Zadnji kraj VI 56. 20. 12. 1973.

Cerkniško jezero: Zadnji kraj, VL56, 20. 12. 1972, B. Drovenik leg.

Lubnik, 1024 m, VM41, 20. 3. 1983, V. Furlan leg. Povir, VL16, 16. 5. 1984, V. Furlan leg.

#### Ischnocoris hemipterus (Schilling, 1829)

Specimens examined:

Kras: Veliki Dol, VL07, 22. 3. 1992, A. & M. Gogala leg. Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

Istra: Dragonja, Stena, UL93, 6. 5. 2000, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 25. 8. 2005 on Calluna vulgaris, A. Gogala leg.

#### Lamproplax picea (Flor, 1860)

A. Gogala, 1991

Specimens examined:

Gorjanci: Sv. Miklavž, WL26, 29. 8. 1990, V. Furlan leg. Ajdovščina, Podčaven, VL18, 7. 1981, B. Drovenik leg.

# Scolopostethus affinis (Schilling, 1829)

Gogala & Moder, 1960: Ljubljana, Šmartno ob Savi, Bohinj; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 15. 3. 1945, Staudacher leg. Medvode, VM51, 17. 3. 1979, A. & M. Gogala leg. Vipava, VL17, 23. 2. 1980, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 16. 8. 1984, 30. 3. 1987, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 18. 2. 1998, M. Gogala leg. Ljubljana, Lavrica, VL69, 23. 4. 1991, V. Furlan leg. Kras: Brje pri Komnu, VL07, 20. 8. 2006, A. Gogala leg. Kresnice, Pogonik, VM80, 29. 7. 1989, V. Furlan leg.

#### Scolopostethus cognatus Fieber, 1861

Kment *et al.*, 2005

Specimen examined:

Kraški rob: Kozina, Kastelec, VL14, 30. 4. 2002, Z. Malinka leg.

#### Scolopostethus decoratus (Hahn, 1833)

Gogala & Gogala, 1989; A. Gogala, 1992

Specimens examined:

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, A. & M. Gogala leg.

Velike Bloke, VL57, 25. 4. 1992, A. & M. Gogala leg. Muljava, Oslica, VL88, 1. 5. 1991, V. Furlan leg. Podkum, Medvedov graben, WM00, 16. 4. 1991, V. Furlan leg.

Ig, Matena, VL69, 24. 4. 1999, S. Brelih leg. Borovak pri Podkumu, WM00, 9. 5. 1990, V. Furlan leg.

# Scolopostethus grandis Horváth, 1880

Scolopostethus pseudograndis Wagner, 1949

Gogala & Moder, 1960: Lubnik; Gogala & Gogala, 1989 Specimens examined:

Postojna, Razdrto, VL26, 6. 5. 1979, A. & M. Gogala leg.

Kraški rob: Bezovica, VL14, 28. 2. 1992, A. & M. Gogala leg.

Cerkniško jezero: Dolenje Jezero, VL56, 25. 4. 1992, A. & M. Gogala leg.

Goričko: Kančevci, WM98, 26. 5. 1997, S. Brelih leg.

#### **Scolopostethus pictus** (Schilling, 1829)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Carniola; Protić, 1987: Podčetrtek, E. Jaeger leg.; Gogala & Gogala, 1989

Specimens examined:

Cerkniško jezero: Dolenje Jezero, VL56, 24. 5. 1987, A. & M. Gogala leg.

Istra: Sečovlje, UL93, 3. 7. 1983, A. & M. Gogala leg. Ljubljana, VM60, 10. 1983, A. & M. Gogala leg.

Sp. Branica, Čipnje, VL07, 25. 5. 1997, A. & M. Gogala leg.

Braniška dolina: Kodreti, Dolanci, VL17, 2. 5. 2000, A. & M. Gogala leg.

Prekmurje: Murski Petrovci, WM86, 11. 4. 1997, S. Brelih leg.

Mirtoviči, VL84, 28. 5. 1988, V. Furlan leg. Ribjek, VL74, 28. 5. 1988, V. Furlan leg.

Zasavje: Podkraj, WM00, 10. 5. 1989, V. Furlan leg. Podkum, Sp. Šklendrovec, WM00, 4. 6. 1988, V. Furlan

Breg, Šentjur na Polju, WM10, 22. 5. 1988, V. Furlan leg.

Kresnice, Ribče, VM80, 18. 5. 1988, V. Furlan leg. Terme Čatež, WL48, 27. 4. 1998, V. Furlan leg.

#### Scolopostethus pilosus Reuter, 1875

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986

Specimens examined:

Laibach (= Ljubljana), 3. 11. 1935, 14. 4. 1940, Staudacher leg.

Bela krajina: Preloka, WL23, 13. 9. 1981, A. & M. Gogala leg.

Dolina Kolpe: Sp. Bilpa – Vrt, 200 m, VL93, 29. 4. 2001, S. Brelih leg.

Brezovica pri Borovnici, VL58, 2. 5. 1997, S. Brelih leg. Ljubljana, Lavrica, VL69, 23. 4. 1991, V. Furlan leg.

#### Scolopostethus puberulus Horváth, 1887

Horváth, 1887: Gorica (type locality); Gogala & Gogala, 1986

Specimens examined:

Logatec, VL48, 13. 4. 1980, A. & M. Gogala leg. Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala leg.

Velike Bloke, VL57, 25. 4. 1992, A. & M. Gogala leg. Ljubljana, Ljubljansko barje, VL69, 24. 2. 1992, 26. 2. 1992, V. Furlan leg.

Kozje, Bistri graben, WL49, 11. 5. 1993, V. Furlan leg. lg, Matena, VL69, 24. 4. 1999, S. Brelih leg.

Kamniško-Savinjske Alpe: Korošica, potok, VM62, 20. 6. 2005, S. Brelih leg.

Ljubljana, Golovec: Orle, VL69, 20. 4. 1984, V. Furlan leg.

#### Scolopostethus thomsoni Reuter, 1875

Gräffe, 1911: Logatec; Gogala & Moder, 1960: Dolnice, Črnuče; Gogala & Gogala, 1986, 1989

Specimens examined:

Ljubljana, Črnuče, VM60, 2. 7. 1933, Staudacher leg. Moravče, Limbarska gora, VM81, 10. 2. 1980, A. & M. Gogala leg.

Turjak, VL78, 25. 5. 1980, A. & M. Gogala leg. Medvode, Goričane, VM51, 15. 7. 1980, A. & M. Gogala leg.

Prekmurje: Moravci, WM97, 5. 7. 1980, A. & M. Gogala leg.

Ljubljansko barje: Bevke, VL59, 27. 3. 1982, A. & M. Gogala leg., 11. 10. 1999, A. Gogala leg.

Petanjci, WM86, 29. 4. 1983, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 10. 8. 1983, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 21. 4. 1984, A. & M. Gogala leg.

Medvode, VM51, 17. 3. 1979, A. & M. Gogala leg. Ig, VL69, 4. 5. 1985, A. & M. Gogala leg.

Julijske Alpe: Krnska jezera, UM92, 31. 7. 1988, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg.

Cerkniško jezero: Dolenje Jezero, VL56, 25. 4. 1992, A. & M. Gogala leg.

Velike Bloke, VL57, 25. 4. 1992, A. & M. Gogala leg. Nanos: Pleša, VL27, 25. 7. 1992, A. & M. Gogala leg. Kum, WM00, 26. 7. 1996, A. & M. Gogala leg., 20. 7. 1987, V. Furlan leg.

Sorica, Soriška planina, VM22, 24. 7. 1991, V. Furlan leg.

Ljubljana, Lavrica, VL69, 8. 5. 1991, 20. 5. 1991, V. Furlan leg.

Mrzlica: Preval Vrhe, WM01, 28. 5. 1991, V. Furlan leg. Postojna, VL37, 10. 6. 1991, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 21. 5. 1991, V. Furlan leg.

Sp. Šklendrovec, WM00, 30. 5. 1991, V. Furlan leg. Radenci, Hrastje-Mota, WM86, 29. 7. 1998, S. Brelih leg.

Gor. Radgona, Črešnjevci, WM76, 24. 4. 1998, S. Brelih leg.

Čaven: mountain chalet, 1240 m, VL18, 27. 5. 1999, S. Brelih leg.

Bovec, Bavšica, UM93, 22. 7. 2000, S. Brelih leg. Ribnica, Ugar, the Ribnica river, VL76, 16. 2. 2001, S. Brelih leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg. Krma, VM14, 14. 5. 1983, V. Furlan leg.

Ljubljana: Zadvor, VL69, 30. 4. 1985, V. Furlan leg. Pohorje: Ribniški vrh, 1500 m, WM25, 20. 7. 1989, V. Furlan leg.

Ljubljana: Šmartno, VM60, 4. 5. 1988, V. Furlan leg.

Gorjanci: Sv. Miklavž, WL26, 29. 8. 1990, V. Furlan leg.

Taphropeltus contractus (Herrich-Schaeffer, 1835)

Horváth, 1887: Gorica; Gogala & Gogala, 1986, 1994 Specimens examined:

Istra: Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Hrastnik, Krnice, WM00, 26. 5. 1997, M. Gogala leg. Ankaran, VL04, 28. 10. 2000, A. & M. Gogala leg. Kras: Brje pri Komnu, VL07, 12. 11. 2005, M. Gogala leg.

Črni Kal, Osp, VL14, 8. 4. 1979, V. Furlan leg.

#### Gonianotini

#### Aphanus rolandri (Linnaeus, 1758)

Gogala & Gogala, 1986, 1989

Specimens examined:

Ljubljansko barje: Podpeč, Jezero, VL59, 4. 4. 1987, A. & M. Gogala leg.

Istra: Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Ljubljana: Žale, VM60, 17. 4. 2000, A. & M. Gogala leg. Podgorski kras: Hrpelje, Prešnica, VL14, 23. 5. 1999, S. Brelih leg.

Koper, Škocjanski zatok, VL04, 23. 4. 2002, S. Brelih leg.

Istra: Zanigrad, VL14, 31. 5. 2004, S. Brelih leg. Kras: Komen, Mali Dol, VL07, 6. 1981, B. Drovenik leg. Ljubljana, VM60, 2. 6. 1984, V. Furlan leg.

#### Emblethis denticollis Horváth, 1878

Péricart, 2001a

Specimen examined:

Kras: Komen, Vale, UL97, 21. 10. 2000, A. & M. Gogala leg.

# **Emblethis griseus** (Wolff, 1802)

Gogala & Moder, 1960: Ljubljana; A. Gogala, 1996 Specimens examined:

Ljubljansko barje: Log, Lukovica, VL59, 17. 4. 1995, A. Gogala leg.

Kras: Brje pri Komnu, VL07, 30. 8. 1998, 28. 7. 2002, A. & M. Gogala leg.

Istra: Strunjan, rt Ronek, UL94, 3. 9. 1998, V. Furlan leg.

#### Emblethis verbasci (Fabricius, 1803)

Montandon, 1886: Gorica; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Ljubljana: Rožnik, VM50, 24. 2. 1954, M. Gogala leg. Senožeče, VL26, 15. 4. 1979, A. & M. Gogala leg. Ljubljansko barje: Ig, Draga, VL68, 8. 8. 1976, S. Brelih leg.

Štorje, VL16, 8. 6. 1983, A. & M. Gogala leg. Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg. Slavnik, VL14, 2. 6. 1984, A. & M. Gogala leg.

Črni kal, VL14, 7. 8. 1986, 1. 11. 1986, 7. 6. 1987, A. & M. Gogala leg.

Brje pri Komnu, VL07, 21. 5. 1989, A. & M. Gogala leg. Istra: Izvir Rižane, VL14, 18. 2. 1990, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 18. 5. 1990, A. & M. Gogala leg.

Kras: Temnica, UL97, 27. 4. 1998, A. & M. Gogala leg. Trstelj, UL98, 25. 6. 2000, A. & M. Gogala leg. Hrpelje, Prešnica, VL14, 7. 6. 1999, S. Brelih leg. Brestovica pri Komnu, Drenovce, UL97, 28. 6. 2005, S.

Škrbina, Vel. Ovčnjak, VL08, 30. 7. 2006, A. Gogala leg.

#### Macrodema microptera (Curtis, 1836)

Gogala & Moder, 1960: Šentvid nad Ljubljano, Črni vrh – Novak (VL29); Gogala & Gogala, 1986

Specimens examined:

Brelih leg.

Prekmurje: Selo, WM97, 5. 7. 1980, A. & M. Gogala leg.

Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris*, A. Gogala leg.

#### Pterotmetus staphyliniformis (Schilling, 1829)

Gogala & Moder, 1960: Ljubljana, Dobrova; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), Golovec, VL69, Stussiner leg. Podčetrtek, WM41, 30. 5. 1933, E. Jaeger leg.

Bela krajina: Adlešiči, WL24, 13. 9. 1981, A. & M. Gogala leg.

Ig, Kurešček, VL68, 2. 5. 1982, A. & M. Gogala leg. Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala leg.

Brkini: Artviže, VL25, 28. 7. 1984, A. & M. Gogala leg. Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, A. & M. Gogala leg.

Prekmurje: Gomilica, XM06, 13. 6. 1987, A. & M. Gogala leg.

Podčetrtek, Vonarje, WM41, 6. 8. 1996, A. Gogala leg. Kraški rob: Zazid, Zalipnik, VL13, 26. 5. 2000, A. Gogala leg.

Bloke: Mramorovo pri Lužarjih, VL67, 15. 8. 2001, A. Gogala leg.

Ljubljansko barje: Goričica, Goriški mah, VL59, 24. 8. 2002, A. Gogala leg.

Ljubljana, Zalog, VM70, 29. 5. 1991, V. Furlan leg. Bela krajina: Stara Lipa, Drežnik, WL13, 30. 6. 1993, V. Furlan leg.

Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris*, A. Gogala leg.

### Trapezonotus anorus (Flor, 1860)

Gogala & Gogala, 1989 Specimens examined: Velike Bloke, VL57, 19. 4. 1987, A. & M. Gogala leg. Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Julijske Alpe: Krma, VM14, 14. 5. 1983, V. Furlan leg.

#### Trapezonotus arenarius (Linnaeus, 1758)

A. Gogala, 1991

Specimens examined:

Istra: Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Vremščica, 800 m, VL26, 11. 6. 2000, A. & M. Gogala leg.

Vremščica, VL26, 10. 5. 1987, V. Furlan leg.

Kraški rob: Zazid, Lipnik, VL13, 7. 7. 2001, A. Gogala leg.

#### Trapezonotus desertus Seidenstücker, 1951

Gogala & Gogala, 1989

Specimens examined:

Julijske Alpe: Komna, VM02, 7. 7. 1983, A. & M. Gogala leg.

Rombon – Pl. Goričica, UM83, 9. – 11. 6. 2000, A. Kapla leg.

Sorica, Soriška planina, 1500 m, VM22, 24. 7. 1991, V. Furlan leg.

#### **Trapezonotus dispar** Stål, 1872

Trapezonotus quadratus auct. (non Fabricius, 1798) Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 15. 9. 1931, 20. 6. 1939, Staudacher leg.

Ljubljana: Ježica, VM60, 1. 5. 1948, Staudacher leg. Škofja Loka, Lubnik, VM41, 7. 1922, M. Hafner leg. Ljubljana: Savlje, VM60, 5. 3. 1977, 15. 4. 1987, A. & M. Gogala leg.

Logatec, VL48, 13. 4. 1980, A. & M. Gogala leg. Slavnik, VL14, 31. 5. 1981, A. & M. Gogala leg. Kočevje, Šalka vas, VL95, 6. 1980, A. & M. Gogala leg. Polhograjsko hrib.: Črni vrh, VM40, 15. 5. 1982, A. & M. Gogala leg.

Ljubljansko barje: Log, Lukovica, VL59, 20. 3. 1983, A. & M. Gogala leg.

Topol, Sv. Katarina, VM50, 21. 4. 1983, M. Gogala leg. Sorica, Soriška planina, VM22, 23. 6. 1984, A. & M. Gogala leg.

Brkini: Artviže, VL25, 28. 7. 1984, A. & M. Gogala leg. Pokljuka: Barje Šijec, VM23, 26. 6. 1985, A. Gogala leg. Rakitna, Pikovnik, VL57, 19. 4. 1987, A. & M. Gogala leg.

Cerkniško jezero: Dolenje Jezero, VL56, 24. 5. 1987, A. & M. Gogala leg.

Pomurje: Veržej, WM86, 13. 6. 1987, A. & M. Gogala leg.

Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg. Idrija, Krekovše, VL19, 28. 6. 1988, M. Gogala leg.

Bela krajina: Vinica, WL23, 3. 6. 1995, M. Gogala leg. Dobrova, Šujica, VM50, 7. 2. 1998, A. & M. Gogala leg. Kras: Trstelj, UL98, 15. 8. 1999, A. & M. Gogala leg. Krim, 800 m, VL58, 31. 3. 1997, S. Brelih leg. Šmartno ob Paki, WM03, 1. 7. 1997, S. Brelih leg. Kum, 1000 m, WM00, 30. 5. 1989, V. Furlan leg., 9. 7. 1987, B. Drovenik leg.

Cerkniško jezero: Goričica, VL56, 10. 6. 1991, V. Furlan leg.

Maribor, Mariborski otok, WM45, 12. 5. 1992, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 20. 4. 1984, 27. 3. 1989, 13. 3. 1991, V. Furlan leg.

Kamniško-Savinjske Alpe: Dolina Korošice, 800 m, VM62, 23. 5. 2001, S. Brelih leg.

Nova Gorica, Panovec, UL98, 10. 5. 2001, S. Brelih leg. Borovec pri Kočevski Reki, Ravne, VL84, 18. 6. 2002, S. Brelih leg.

Javorniki: Vršič, 700 m, VL46, 7. 6. 2001, S. Brelih leg. Banjaloka, Stružnica, VL83, 24. 4. 2001, S. Brelih leg. Gornji lg, 600 m, VL68, 5. 6. 1982, V. Furlan leg. Ratitovec, 1100 m, VM22, 6. 6. 1982, V. Furlan leg. Luče, Igla, VM73, 30. 5. 1983, B. Drovenik leg. Savinjske Alpe: Luče, Veža-Planica, VM73, 7. 1983, B. Drovenik leg.

Krka, VL88, 7. 6. 1987, S. Brelih leg.

Kamniške Alpe: Okrešelj, 1400 m, VM63, 1. 7. 1992, V. Furlan leg.

Kranjska Gora, Mala Pišnica, VM04, 11. 6. 1988, V. Furlan leg.

Osilnica, VL74, 28. 5. 1988, V. Furlan leg. Idrijska Bela, Belca, VL19, 24. 7. 1988, V. Furlan leg. Podkum, Sopota, WM00, 28. 3. 1989, V. Furlan leg. Ig, Iška vas, VL68, 3. 5. 1987, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg.

#### *Trapezonotus ullrichi* (Fieber, 1837)

Reuter, 1888: Gorica; Gogala & Moder, 1960: Šmartno ob Savi; Protić, 1987: Podčetrtek, E. Jaeger leg.; Gogala & Gogala, 1989

Specimens examined:

Ljubljansko barje: Log, Lukovica, VL59, 22. 11. 1987, A. Gogala leg.

Kras: Brje pri Komnu, VL07, 18. 6. 1989, A. & M. Gogala leg.

Kozina, VL15, 22. 6. 1991, V. Furlan leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, S. Brelih leg. Rodik, 600 m, VL15, 7. 6. 2001, S. Brelih leg.

#### Megalonotini

#### Lamprodema maura (Fabricius, 1803)

Péricart, 2001a

Specimen examined:

Istra: Ankaran, VL04, 28. 10. 2000, A. & M. Gogala leg.

#### Lasiocoris anomalus (Kolenati, 1845)

Montandon, 1886: Gorica; A. Gogala, 1991; Gogala & Gogala, 1994

Specimens examined:

Sočerga, Veli Badin, VL13, 1. 8. 1990, A. & M. Gogala leg.

Istra: Movraž, VL13, 14. 6. 1991, A. & M. Gogala leg. Kozina, Prešnica, VL14, 22. 6. 1991, A. & M. Gogala leg.

Kras: Brestovica pri Komnu, UL97, 1. 6. 1996, A. & M. Gogala leg.

#### Megalonotus antennatus (Schilling, 1829)

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg. Specimens examined:

Laibach (= Ljubljana), 29. 9. 1928, 30. 9. 1928, 16. 2. 1930, Staudacher leg.

Tržič, Visoče, VM43, 28. 3. 1981, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 30. 5. 1982, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 15. 5. 1982, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 21. 4. 1984, A. & M. Gogala leg.

Vransko, Motnik, VM91, 9. 5. 1987, S. Brelih leg.

Kras: Veliki Dol, VL07, 22. 3. 1992, A. & M. Gogala leg. Vremščica, VL26, 5. 7. 1999, A. Gogala leg.

Ljubljana, Ljubljansko barje, VL69, 26. 2. 1992, V. Furlan leg.

Kamniško-Savinjske Alpe: Dolina Korošice, 800 m, VM62, 23. 5. 2001, S. Brelih leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 15. 5. 1983, 4. 5. 1984, V. Furlan leg.

Mali Kum, WM00, 6. 5. 1988, V. Furlan leg.

#### Megalonotus chiragra (Fabricius, 1794)

Horváth, 1887: Gorica; Gogala & Moder, 1960, Gogala & Gogala, 1986, 1989: partly confused with other species

Specimens examined:

Laibach (= Ljubljana), 29. 9. 1928, Staudacher leg. Pokojišče, VL58, 27. 4. 1930, Staudacher leg.

Radovljica, Brezje, VM43, 24. 4. 1979, A. & M. Gogala leg.

Log, Lukovica, VL59, 20. 3. 1983, 20. 7. 1983, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 21. 4. 1984, 15. 4. 1987, 11. 4. 1988, A. & M. Gogala leg.

Ljubljansko barje: Ig, VL69, 4. 5. 1985, A. & M. Gogala leg.

Zg. Radovna, VM14, 28. 8. 1988, A. & M. Gogala leg. Vrhnika, Drenov grič, VL49, 18. 2. 1989, M. Gogala leg. Bloke: Mramorovo pri Lužarjih, VL67, 15. 8. 2001, A. Gogala leg.

Logatec, Zaplana: Jezerce, VL38, 27. 4. 2005, A. Gogala leg.

Pokojišče, Zavrh, VL48, 9. 6. 2005, A. Gogala leg. Novo mesto, Trška gora, WL17, 19. 6. 1993, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 21. 5. 1991, V. Furlan leg.

Prekmurje: Murski Petrovci, WM86, 31. 7. 1998, 1. 8. 1998, S. Brelih leg.

Ig, Kremenica, VL68, 8. 3. 1999, S. Brelih leg. Ljubljana: Šiška, VM60, 27. 6. 1999, S. Brelih leg. Polhograjsko hrib.: Grmada, VM40, 6. 5. 1984, V. Furlan leg.

Topol, Osredek, VM50, 3. 6. 1984, V. Furlan leg.

#### Megalonotus dilatatus (Herrich-Schaeffer, 1840)

Montandon, 1886: Gorica; Gräffe, 1911: Klanec pri Kozini; Gogala & Gogala, 1986, 1989; A. Gogala *et al.*, 1990

Specimens examined:

Brkini: Artviže, VL25, 28. 7. 1984, A. & M. Gogala leg. Bohinj: Ukanc, VM02, 24. 7. 1987 on *Genista*, 4. 10. 1987, A. & M. Gogala leg.

Istra: Strunjan, UL94, 17. 9. 1989, A. & M. Gogala leg. Dragonja, UL93, 22. 7. 1997, A. & M. Gogala leg. Kras: Trstelj, UL98, 25. 6. 2000, A. & M. Gogala leg. Kraški rob: Zazid, Lipnik, VL13, 7. 7. 2001, A. Gogala leg.

# Megalonotus emarginatus (Rey, 1888)

A. Gogala, 1991; Péricart, 1998c: Istra Specimens examined:

Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg.

Kras: Komen, Kregolišče, VL07, 19. 4. 1992, A. Gogala leg.

Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

Sečovlje, Fontanigge, UL93, 6. 5. 2000, A. Gogala leg. Istra: Popetre, VL03, 9. 7. 1997, S. Brelih leg.

Muljava, VL88, 3. 3. 1992, V. Furlan leg.

Novo mesto, Trška gora, WL17, 7. 5. 1992, V. Furlan leg.

Koper, Škocjanski zatok, VL04, 23. 5. 2000, S. Brelih leg.

Dragonja, UL93, 4. 5. 2000, S. Brelih leg.

Bela krajina: Stranska vas, WL15, 28. 4. 1983, V. Furlan leg.

#### Megalonotus hirsutus Fieber, 1861

Horváth, 1887: Gorica; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Ljubljana, Medno, 25. 5. 1931, M. Hafner leg. Log, Lukovica, VL59, 14. 5. 1983, 14. 7. 1989, A. & M. Gogala leg. Ljubljansko barje: Notranje Gorice, VL59, 31. 5. 1987, A. & M. Gogala leg.

Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg.

Ig, Kremenica, VL68, 5. 6. 2002, S. Brelih leg.

Bela krajina: Semič, Gornja Paka, WL15, 29. 4. 1983, V. Furlan leg.

Gornji Ig, VL68, 23. 5. 1987, V. Furlan leg.

#### Megalonotus praetextatus (Herrich-Schaeffer, 1835)

A. Gogala, 1991

Specimens examined:

Kras: Brje pri Komnu, VL07, 9. 7. 1989, 1. 8. 2005, A. & M. Gogala leg.

Vipavska dolina: Ozeljan, Lijak, UL99, 8. 4. 1992, S. Brelih leg.

Sočerga, Veli Badin, VL13, 27. 6. 1995, 20. 5. 2001, A. & M. Gogala leg.

Istra: Zanigrad, VL14, 7. 7. 2003, S. Brelih leg. Gorenje pri Divači, VL16, 26. 5. 2004, S. Brelih leg.

#### Megalonotus sabulicola (Thomson, 1870)

Montandon, 1886: Gorica; A. Gogala, 1991; Gogala & Gogala, 1994

Specimens examined:

Črni kal, VL14, 30. 6. 1979, A. & M. Gogala leg.

Kras: Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg.

Brje pri Komnu, VL07, 9. 8. 1995, M. Gogala leg., 30. 8. 1998, 26. 4. 2000, A. & M. Gogala leg.

Tublje pri Komnu, VL07, 26. 4. 1998, A. & M. Gogala leg.

Braniška dolina: Kodreti, Dolanci, VL17, 2. 5. 2000, A. & M. Gogala leg.

Dragonja, UL93, 4. 5. 2000, S. Brelih leg.

Nova Gorica, Panovec, UL98, 23. 4. 2000, B. Zadravec leg.

#### Sphragisticus nebulosus (Fallén, 1807)

Gogala & Gogala, 1986

Specimen examined:

Prekmurje: Dobrovnik, XM06, 23. 7. 1983, A. & M. Gogala leg.

# Myodochini

# Pachybrachius fracticollis (Schilling, 1829)

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 16. 2. 1930, 14. 4. 1940, Staudacher leg.

Pijava gorica, Rogovila, VL68, 24. 5. 1981, A. & M. Gogala leg.

Ljubljansko barje: Bevke, VL59, 17. 4. 1983, A. & M. Gogala leg.

Postojna, Landol, VL37, 21. 9. 1983, A. & M. Gogala leg.

Horjul, Lesno brdo, VL49, 8. 6. 1986, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, 10. 9. 1988, A. & M. Gogala leg.

Cerkniško jezero: Dolenje Jezero, VL56, 24. 5. 1987, A. & M. Gogala leg.

Cerkniško jezero: Gorenje Jezero, VL56, 23. 8. 2002, A. Gogala leg.

Bizeljsko, Stara vas, WL59, 21. 4. 2000, A. Gogala leg. Moravče, Prikrnica, ob Drtijščici, VM71, 19. 5. 1997, S. Brelih leg.

Cerkniško jezero: Zadnji kraj, VL56, 22. 5. 1991, S. Brelih leg.

Cerkniško jezero: Goričica, VL56, 10. 6. 1991, V. Furlan leg.

Novo mesto, Trška gora, WL17, 11. 6. 1992, V. Furlan leg.

Ljubljana: Dobrunje – Sv. Urh, VL69, 25. 6. 1997, V. Furlan leg.

Slovenske gorice: Savci, WM84, 23. 4. 1998, S. Brelih

Prekmurje: Bukovnica, XM07, 2. 6. 1999, S. Brelih leg. Ljubljana, Ljubljansko barje, VL69, 12. 5. 1979, 29. 4. 1980, 31. 5. 1980, 1. 6. 1980, V. Furlan leg.

#### Pachybrachius luridus Hahn, 1826

Gogala & Gogala, 1986, 1989

Specimens examined:

Ljubljana, Ljubljansko barje, VL69, 1. 5. 1980, 7. 5. 1981, 10. 5. 1985, V. Furlan leg.

#### Paraparomius leptopoides (Baerensprung, 1859)

Montandon, 1886: Gorica; Gogala & Gogala, 1989; Péricart, 1998c: Istra: Dragonja

Specimens examined:

Istra: Portorož, UL94, 15. 10. 1986, A. & M. Gogala leg. Nova Gorica, Panovec, UL98, 15. 5. 2000, S. Brelih leg.

### Paromius gracilis (Rambur, 1839)

Montandon, 1886: Gorica; Gogala & Gogala, 1986, 1989; A. Gogala *et al.*, 1990

Specimens examined:

Istra: Padna, UL93, 4. 11. 1983, 19. 8. 1996, 28. 10. 2000, A. & M. Gogala leg.

Portorož, UL94, 5. 11. 1986, M. Gogala leg., 1. 10. 1987, A. & M. Gogala leg.

Strunjan, UL94, 17. 2. 1988, A. & M. Gogala leg.

Dragonja, Stena, UL93, 30. 8. 1989, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

Komen, Vale, UL97, 21. 10. 2000, A. & M. Gogala leg. Nova Gorica, Panovec, UL98, 15. 5. 2000, 6. 7. 2000, S. Brelih leg.

Strunjan, rt Ronek, UL94, 3. 9. 1998, 4. 9. 1998, 6. 9. 1998, V. Furlan leg.

#### Plinthisini

# Plinthisus pusillus (Scholtz, 1847)

Horváth, 1887: Gorica

#### *Plinthisus brevipennis* (Latreille, 1807)

Horváth, 1887: Gorica; Gogala & Gogala, 1986 Specimens examined:

Laibach (= Ljubljana), 3. 6. 1944, Staudacher leg.

Ocizla, VL15, 8. 4. 1979, E. Pretner leg.

Brje pri Komnu, VL07, 7. 9. 1989, 19. 4. 1992, 10. 10. 1999, 30. 6. 2002, A. & M. Gogala leg., 12. 11. 2005, M. Gogala leg.

Kras: Veliki Dol, VL07, 22. 3. 1992, A. & M. Gogala leg. Gorjansko, UL97, 27. 4. 1992, A. & M. Gogala leg. Slavnik, VL14, 19. 6. 1995, M. Gogala leg.

Komen, Vale, UL97, 10. 10. 1999, A. & M. Gogala leg. Istra: Dragonja, Stena, UL93, 6. 5. 2000, A. & M. Gogala leg.

Trstelj, UL98, 25. 6. 2000, A. & M. Gogala leg. Kras: Škocjan, Naklo, right bank of the Reka river, VL25, 11. 10. 2001, A. Pirnat leg.

#### Plinthisus longicollis Fieber, 1861

Plinthisus brevicollis Ferrari, 1874

Plinthisus hungaricus Horváth, 1875

Montandon, 1886: Gorica; Horváth, 1887: Gorica; Gogala & Moder, 1960: Črni kal; Gogala & Gogala, 1989 Specimens examined:

Istra: Dragonja, Stena, UL93, 1. 10. 1987 under *Sedum acre*, A. & M. Gogala leg.

Sočerga, Gradec, VL13, 28. 10. 2000, A. Gogala leg.

#### Rhyparochromini

#### Aellopus atratus (Goeze, 1778)

Reuter, 1888: Gorica; Gogala & Moder, 1960: Gorica; Gogala & Gogala, 1986, 1994

Specimens examined:

Istra: Sočerga, Veli Badin, VL13, 9. 6. 1990, A. & M. Gogala leg.

# Beosus maritimus (Scopoli, 1763)

Beosus luscus (Fabricius, 1794)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana, Črni kal, Piran; Gogala & Gogala, 1986, 1989, 1994; A. Gogala *et al.*, 1990

Specimens examined:

Ljubljana, Tacen, VM50, 2. 3. 1980, A. & M. Gogala leg.

Sečovlje, UL93, 20. 9. 1980, A. & M. Gogala leg.

Ljubljansko barje: Vrhnika, Log, VL59, 7. 3. 1981, A. & M. Gogala leg.

Bela krajina: Preloka, WL23, 13. 9. 1981, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 13. 3. 1983, 19. 4. 1984, 11. 4. 1988, A. & M. Gogala leg.

Istra: Boršt, VL03, 3. 5. 1986, A. & M. Gogala leg. Vrhnika, Drenov grič, VL49, 18. 2. 1989, M. Gogala leg. Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg.

Kras: Brje pri Komnu, VL07, 7. 9. 1989, A. & M. Gogala leg.

Strunjan, UL94, 17. 9. 1989, A. & M. Gogala leg. Kraški rob: Socerb, VL14, 1. 8. 1990, A. & M. Gogala leg.

Nova Gorica, Kromberk, UL98, 2. 10. 1990, M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 6. 5. 2000, A. Gogala leg.

Ljubljana: Žale, VM60, 17. 4. 2000, A. & M. Gogala leg. Log, Lukovica, VL59, 8. 2. 2001, A. & M. Gogala leg.

#### Beosus quadripunctatus (Müller, 1766)

Gogala & Moder, 1960: Šmartno ob Savi; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Istra: Koštabona, VL03, 25. 6. 1981, M. Gogala leg. Ankaran, VL04, 8. 6. 1983, A. & M. Gogala leg. Kraški rob: Črni kal, VL14, 22. 10. 1983, M. Gogala leg. Padna, UL93, 16. 6. 1984, A. & M. Gogala leg. Portorož, Beli križ, UL84, 10. 10. 1984, M. Gogala leg. Istra: Boršt, VL03, 3. 5. 1986, A. & M. Gogala leg. Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Sočerga, Veli Badin, VL13, 1. 8. 1990, A. & M. Gogala leg.

Nova Gorica, Kromberk, UL98, 2. 10. 1990, M. Gogala leg.

Dragonja, UL93, 22. 7. 1997, A. & M. Gogala leg., 4. 5. 2000, S. Brelih leg.

Kras: Brje pri Komnu, VL07, 13. 2. 2000, A. & M. Gogala leg.

Koper, Škocjanski zatok, VL04, 30. 5. 2002, S. Brelih leg.

Strunjan, rt Ronek, UL94, 4. 9. 1998, V. Furlan leg.

#### Graptopeltus lynceus (Fabricius, 1775)

Horváth, 1887: Gorica; Gogala & Moder, 1960: Ljubljana, Šmartno ob Savi; Gogala & Gogala, 1986, 1989 Specimens examined:

Laibach (= Ljubljana), 29. 9. 1928, 15. 10. 1936, Staudacher leg.

Radovljica, Brezje, VM43, 24. 4. 1979, A. & M. Gogala leg.

Ljubljana: Šentvid, VM50, 8. 2. 1970, B. Drovenik leg. Ljubljana, Tacen, VM50, 2. 3. 1980, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 13. 3. 1983, 19. 4. 1984, A. & M. Gogala leg.

Ljubljansko barje: Log, Lukovica, VL59, 20. 3. 1983, 1. 4. 1988, A. & M. Gogala leg.

Goričko: Gornji Petrovci, WM98, 1. 5. 1983, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 10. 8. 1983, A. & M. Gogala leg.

Dolina Raše: Griže, VL16, 21. 3. 1992, A. & M. Gogala leg.

Cerkniško jezero: Dolenje Jezero, VL56, 25. 4. 1992, A. & M. Gogala leg.

Ig, VL69, 10. 2. 1974, V. Furlan leg.

Zasavje: Kum, 1000 m, WM00, 30. 5. 1989, V. Furlan leg.

#### *Graptopeltus validus* (Horváth, 1875)

Graptopeltus consors (Horváth, 1878)

Gogala & Gogala, 1989; A. Gogala, 1991

Specimen examined:

Novo mesto, Trška gora, WL17, 21. – 22. 5. 1983, V. Furlan leg.

#### Panaorus adspersus (Mulsant & Rey, 1852)

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 15. 9. 1935, Staudacher leg. Ljubljansko barje: Log, Lukovica, VL59, 12. 9. 1987, A. Gogala leg.

Suha krajina: Žvirče, VL87, 7. 5. 1983, V. Furlan leg. Bela krajina: Preloka, WL23, 19. 4. 1989, V. Furlan leg.

# Peritrechus geniculatus (Hahn, 1832)

Gogala & Moder, 1960: Ljubljana, Ig; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg. Specimens examined:

Laibach (= Ljubljana), 27. 10. 1937, 4. 6. 1938, Staudacher leg.

Studenec – Ig, VL69, 25. 8. 1939, Staudacher leg.

Ljubljana, Tacen, VM50, 2. 3. 1980, A. & M. Gogala leg.

Ljubljansko barje: Plešivica, VL59, 11. 3. 1983, A. & M. Gogala leg.

Log, Lukovica, VL59, 18. 9. 1983 under *Thymus*, 8. 2. 2001, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 15. 4. 1987, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, A. & M. Gogala leg.

Prekmurje: Petišovci, XM15, 13. 6. 1987, A. & M. Gogala leg.

Sp. Brnik, VM61, 7. 9. 1988, A. & M. Gogala leg.

Slavnik, VL14, 23. 6. 1991, V. Furlan leg.

Loški potok, VL66, 8. 6. 1997, V. Furlan leg.

Ig, Kurešček, VL68, 5. 6. 1983, 11. 5. 1990, V. Furlan leg.

Ljubljana, VM60, 6. 6. 1986, V. Furlan leg.

Polhov Gradec, VM40, 6. 5. 1984, V. Furlan leg.

Cerkniško jezero: Dol. Jezero, Goričica, VL56, 27. 6. 1984, V. Furlan leg.

Senožeče, Gabrče, VL26, 26. 5. 1987, V. Furlan leg. Trnovski gozd: Lokve, VL09, 27. 6. 1998, V. Furlan leg.

#### Peritrechus gracilicornis Puton, 1877

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989, 1994; Péricart, 1998c: Sežana, Orlek Specimens examined:

Laibach (= Ljubljana), 8. 9. 1937, 27. 10. 1937, Staudacher leg.

Koper, Škocjanski zatok, VL04, 1. 7. 1979, A. & M. Gogala leg., 23. 5. 2000, 30. 5. 2002, S. Brelih leg. Koper, Sermin, VL04, 16. 6. 1984, A. & M. Gogala leg. Črni kal, VL14, 28. 6. 1980, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 27. 3. 1983, A.

Ljubljansko barje: Log, Lukovica, VL59, 27. 3. 1983, A & M. Gogala leg.

Kras: Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg., 30. 5. 1982, V. Furlan leg.

Istra: Koštabona, VL03, 7. 6. 1987, A. & M. Gogala leg. Brje pri Komnu, VL07, 5. 5. 1989, 16. 8. 2006, A. & M. Gogala leg.

Brestovica pri Komnu, UL97, 2. 5. 1990, A. & M. Gogala leg.

Nova Gorica, Kromberk, UL98, 2. 10. 1990, M. Gogala leg.

Dolina Raše: Griže, VL16, 21. 3. 1992, A. & M. Gogala leg.

Dolina Branice: Dolanci, VL17, 13. 2. 2000, A. & M. Gogala leg.

Padna, UL93, 28. 10. 2000, A. & M. Gogala leg. Kozina, VL15, 22. 6. 1991, V. Furlan leg.

Komen, Branik, VL07, 27. 5. 1998, S. Brelih leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, 13. 9. 2000, 10. 5. 2001, S. Brelih leg.

Obrov, Golac, VL24, 8. 6. 2000, S. Brelih leg. Bela krajina: Preloka, WL23, 27. 4. 1983, V. Furlan leg. Bela krajina: Gradac, WL15, 28. 4. 1983, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 10. 4. 1989, V. Furlan leg.

Šmarješke toplice, WL18, 1. 8. 1998, V. Furlan leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

#### Peritrechus lundii (Gmelin, 1790)

Gogala & Moder, 1960: Ljubljana, Podutik, Šmartno ob Savi; Gogala & Gogala, 1986, 1989 Specimens examined:

Laibach (= Ljubljana), Stussiner leg, 15. 10. 1936, Staudacher leg.

Laibach (= Ljubljana), Golovec, VL69, Stussiner leg. Ljubljana, Podutik, VM50, 25. 3. 1933, Staudacher leg. Bohinj: Ribčev laz, VM12, 20. 7. 1932, M. Hafner leg. Ljubljana: Savlje, VM60, 19. 4. 1984, A. & M. Gogala leg. Ljubljana, Golovec: Orle, VL69, 21. 4. 1985, V. Furlan leg.

#### Peritrechus nubilus (Fallén, 1807)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Dobrova

#### Raglius alboacuminatus (Goeze, 1778)

Gogala & Moder, 1960: Šmartno ob Savi, Bohinj, Črni kal; Gogala & Gogala, 1986, 1989, 1994; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Vrhnika, Log, VL59, 7. 3. 1981, A. & M. Gogala leg. Log, Lukovica, VL59, 20. 3. 1983, A. & M. Gogala leg. Bevke, VL59, 17. 4. 1983, A. & M. Gogala leg. Ljubljana: Savlje, VM60, 21. 4. 1984, 11. 4. 1988, A. & M. Gogala leg.

Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg. Ljubljansko barje: Dragomer, VL59, 1. 1. 1984, A. & M. Gogala leg.

Črni kal, VL14, 7. 6. 1987, A. & M. Gogala leg. Bela krajina: Drašiči, Babna gora, WL25, 13. 8. 1988, M. Štangelj leg.

Vrhnika, Drenov grič, VL49, 18. 2. 1989, M. Gogala leg. Štorje, VL16, 26. 3. 1989, A. & M. Gogala leg. Istra: Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Komen, VL07, 5. 8. 1989, A. & M. Gogala leg. Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Kras: Gorjansko, UL97, 17. 9. 2000, A. & M. Gogala leg.

Kraški rob: Zazid, Lipnik, VL13, 7. 7. 2001, A. Gogala leg.

Brje pri Komnu, VL07, 24. 9. 2006, A. & M. Gogala leg. Hrpelje, Prešnica, VL14, 10. 5. 1999, S. Brelih leg. Ankaran, Valdoltra, VL04, 23. 4. 2002, S. Brelih leg. Ljubljana, Golovec: Orle, VL69, 23. 4. 1983, 15. 4. 1984, 13. 4. 1985, 21. 5. 1991, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. Ljubljana: Polje, Slape, VM60, 7. 5. 1988, 11. 4. 1987, 20. 3. 1991, V. Furlan leg.

Gornji Ig, VL68, 14. 5. 1991, V. Furlan leg.

#### Raglius confusus (Reuter, 1886)

Gogala & Gogala, 1986, 1994; A. Gogala *et al.*, 1990 Specimens examined:

Portorož, UL94, 7. 1. 1979, A. & M. Gogala leg. Bela krajina: Vinica, Zilje, WL23, 13. 9. 1981, A. & M. Gogala leg.

Istra: Padna, UL93, 16. 6. 1984, 1. 2. 1997, A. & M. Gogala leg.

Portorož, Beli križ, UL84, 10. 10. 1984, M. Gogala leg. Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Strunjan, UL94, 17. 9. 1989, A. & M. Gogala leg. Strunjan, Karbonar, UL94, 17. 5. 2003, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg., 24. 7. 1990, V. Furlan leg.

Koper, Škocjanski zatok, VL04, 23. 5. 2000, S. Brelih leg.

Istra: Zanigrad, VL14, 7. 7. 2003, S. Brelih leg.

#### Rhyparochromus phoeniceus (Rossi, 1794)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana, Dobrova, Šmartno ob Savi, Tribuče, Bohinj, Svinjak, Črni kal; Gogala & Gogala, 1986, 1989, 1994 Specimens examined:

Wochein (= Bohinj), VM12, 29. 6. 1930, Staudacher leg. Bohinj: Ribčev laz, VM12, 25. 5. 1934, M. Hafner leg. Slavnik, VL14, 2. 7. 1982, A. & M. Gogala leg., 24. – 25. 5. 1975, 26. 3. – 17. 4. 1977, V. Furlan leg. Medvode, Babni dol, VM50, 22. 6. 1983, A. & M. Gogala leg.

Čaven, VL08, 11. 6. 1988, 22. 8. 1992, A. & M. Gogala leg

Kras: Trstelj, UL98, 19. 8. 1990, A. & M. Gogala leg. Vremščica, VL26, 19. 3. – 2. 4. 1977, 16. 4. – 1. 5. 1977, 18. 4. 1987, 29. 4. 1987, V. Furlan leg.

#### Rhyparochromus pini (Linnaeus, 1758)

Cimex collinus Scopoli, 1763

Scopoli, 1763: Carniolia media (= Notranjska); Gräffe, 1911: Tolmin; Gogala & Moder, 1960; Gogala & Gogala, 1986, 1989

Specimens examined:

Laibach (= Ljubljana), 3. 11. 1935, Staudacher leg. Medvode, Pirniče, VM51, 13. 2. 1977, A. & M. Gogala leg.

Ljubljana, Tacen, VM50, 22. 10. 1976, A. & M. Gogala leg.

Moravče, Limbarska gora, VM81, 10. 2. 1980, A. & M. Gogala leg.

Ljubljana: Šiška, VM50, 16. 2. 1980, A. & M. Gogala leg.

Celje, Griže, WM12, 20. 8. 1969, I. Sivec leg.

Polhov Gradec, VM40, 15. 11. 1981, A. & M. Gogala leg.

Ljubljansko barje: Bevke, VL59, 27. 3. 1982, A. & M. Gogala leg.

Log, Lukovica, VL59, 25. 7. 1982, A. & M. Gogala leg. Vrhnika, Log, VL59, 5. 2. 1983, A. & M. Gogala leg. Ljubljana: Savlje, VM60, 13. 3. 1983, A. & M. Gogala leg.

Velike Bloke, 2 km E, VL67, 24. 8. 1985, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 19. 4. 1987, A. & M. Gogala leg.

Rakek, Unec, VL47, 14. 2. 1988, A. & M. Gogala leg. Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg. Zg. Radovna, VM14, 28. 8. 1988, A. & M. Gogala leg. Vremščica, VL26, 9. 5. 1992, A. & M. Gogala leg. Osredek pri Dobrovi, VM50, 7. 2. 1998, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 25. 8. 2005 on *Calluna vulgaris*, A. Gogala leg.

Bistrica ob Sotli, WM50, 18. 5. 2000, S. Brelih leg. Banjaloka, Stružnica, VL83, 18. 6. 2002, S. Brelih leg. Slavnik, VL14, 26. 3. – 17. 4. 1977, V. Furlan leg. Vremščica, VL26, 19. 3. – 2. 4. 1977, V. Furlan leg. Rakov Škocjan, VL47, 5. 3. 1977, V. Furlan leg. Logatec, VL48, 17. 2. 1974, V. Furlan leg. Muljava, VL88, 24. 8. 1982, 13. 3. 1983, 11. 5. 1985, V. Furlan leg.

Bela krajina: Vinica, WL13, 29. 4. 1983, V. Furlan leg. Bela krajina: Semič, WL15, 30. 4. 1983, V. Furlan leg. Bela krajina: Stranska vas, WL15, 28. 4. 1983, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 23. 4. 1983, 4. 5. 1984, 26. 3. 1985, 27. 3. 1989, 10. 4. 1989, 13. 3. 1991, V. Furlan leg.

Šmihel, Mozirska planina, VM93, 7. 1983, B. Drovenik leg.

Gornji Ig, VL68, 23. 5. 1987, V. Furlan leg. Zasavje: Kum, WM00, 9. 7. 1987, B. Drovenik leg. Savinjske Alpe: Smrekovec, VM94, 26. 6. 1987, B. Drovenik leg.

Gorjanci: Sv. Miklavž, WL26, 29. 8. 1990, V. Furlan leg. Mozirje, Šmihel, VM93, 14. 8. 1998, V. Furlan leg. Suha krajina: Ambrus, VL87, 7. 5. 1983, V. Furlan leg.

# **Rhyparochromus sanguineus** (Douglas & Scott, 1868) Specimens examined:

Črni kal, VL14, 1. 7. 1979, A. & M. Gogala leg. Kras: Trstelj, UL98, 13. 8. 1989, A. & M. Gogala leg. Brje pri Komnu, VL07, 1. 5. 1990, A. & M. Gogala leg. Črni kal, Osp, VL14, 8. 4. 1979, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 27. 3. 1989, V. Furlan leg.

Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

Note: In my opinion, *Rhyparochromus sanguineus* (Douglas & Scott, 1868) is most probably a synonym for *R. phoeniceus*. All characters used by Rieger (1993) to distinguish between the two are very variable in specimens of the same population and in all characters intermediate states are observed.

#### **Rhyparochromus vulgaris** (Schilling, 1829)

Reuter, 1888: Gorica; Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.

Specimens examined:

Laibach (= Ljubljana), 2. 11. 1931, Staudacher leg. Gabrovka, VL99, 1. 12. 1980, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 4. 9. 1982, 4. 4. 1983, A. & M. Gogala leg.

Krka, VL88, 12. 8. 1984, A. & M. Gogala leg.

Pomurje: Veržej, WM86, 13. 6. 1987, A. & M. Gogala leg.

Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

Kras: Štorje, VL16, 26. 3. 1989, A. & M. Gogala leg. Brje pri Komnu, VL07, 16. 9. 1989, A. & M. Gogala leg. Nova Gorica, Kromberk, UL98, 2. 10. 1990, M. Gogala leg.

Novo mesto, Trška gora, WL17, 6. 6. 1987, 7. 5. 1992, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 21. 4. 1985, V. Furlan leg.

Ljubljana: Polje, Slape, VM60, 4. 4. 1987, 7. 5. 1988, 20. 3. 1991, V. Furlan leg.

Polhov Gradec, VM40, 17. 4. 1983, V. Furlan leg. Bela krajina: Podzemelj, WL25, 28. 4. 1983, V. Furlan leg.

Bela krajina: Stranska vas, WL15, 28. 4. 1983, V. Furlan leg.

#### Xanthochilus quadratus (Fabricius, 1798)

Rhyparochromus immaculatus (Royer, 1919)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989, 1994 Specimens examined:

Laibach (= Ljubljana), 8. 9. 1937, Staudacher leg. Črni kal, VL14, 28. 6. 1980, 8. 6. 1983, A. & M. Gogala leg.

Kras: Štorje, VL16, 22. 7. 1980, A. & M. Gogala leg. Senožeče, VL26, 28. 6. 1982, M. Gogala leg. Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg., 30. 5.

1982, 25. 5. 1985, V. Furlan leg.

Brkini: Barka, VL25, 28. 7. 1984, A. & M. Gogala leg. Ljubljansko barje: Podpeč, Jezero, VL59, 4. 4. 1987, A. & M. Gogala leg.

Istra: Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Brje pri Komnu, VL07, 12. 6. 1989, M. Gogala leg., 21. 7. 1990, A. & M. Gogala leg.

Trstelj, UL98, 19. 8. 1990, A. & M. Gogala leg.

Kraški rob: Bezovica, VL14, 3. 10. 1990, A. & M. Gogala leg.

Dolina Raše: Griže, VL16, 21. 3. 1992, A. & M. Gogala leg.

Vremščica, VL26, 4. 7. 1992, A. & M. Gogala leg. Log, Lukovica, VL59, 17. 4. 1995, 8. 2. 2001, A. & M. Gogala leg.

Nanos: Šembijska bajta, VL27, 10. 8. 1996, A. & M. Gogala leg.

Komen, Vale, UL97, 21. 10. 2000, A. & M. Gogala leg. Obrov, Golac, VL24, 8. 6. 2000, S. Brelih leg. Kozina, VL15, 22. 6. 1991, V. Furlan leg.

#### Xanthochilus saturnius (Rossi, 1790)

A. Gogala, 1991

Specimens examined:

Šmartno na Pohorju, WM44, 1989, Macarol leg.

Istra: Strunjan, Belvedere, UL94, 22. 7. 2000, A. Gogala leg.

Strunjan, rt Ronek, UL94, 6. 9. 1998, V. Furlan leg.

#### Stygnocorini

#### **Acompus rufipes** (Wolff, 1804)

Gogala & Moder, 1960: Ljubljana, Kot, Goričane; Gogala & Gogala, 1986, 1989; Protić, 1987: Podčetrtek, E. Jaeger leg.; Péricart, 1998b: Postojna

Specimens examined:

Laibach (= Ljubljana), 31. 5. 1937, Staudacher leg. Ljubljana: Vič, VM50, 19. 4. 1936, Staudacher leg. Medvode, Goričane, VM51, 23. 6. 1938, Staudacher leg.

Kottal (= dolina Kot), VM14, 8. 6. 1935, Staudacher leg. Dobrova, VM50, 27. 5. 1979, A. & M. Gogala leg. Ljubljansko barje: Bevke, VL59, 14. 6. 1980, 26. 8. 1980, A. & M. Gogala leg.

Pijava gorica, Rogovila, VL68, 24. 5. 1981, A. & M. Gogala leg.

Cerkniško jezero: Cerknica, Dolenje Jezero, VL56, 29. 6. 1983, A. & M. Gogala leg.

Julijske Alpe: Komna, VM02, 7. 7. 1983, A. & M. Gogala leg.

Log, Lukovica, VL59, 16. 8. 1983, A. & M. Gogala leg. Polhograjsko hrib.: Črni vrh, Pasja ravan, VM40, 4. 6. 1985, A. & M. Gogala leg.

Velike Bloke, 2 km E, VL67, 24. 8. 1985, A. & M. Gogala leg.

Rakitna, VL58, 22. 6. 1986, A. & M. Gogala leg. Čaven, VL08, 11. 6. 1988, A. & M. Gogala leg. Logarska dolina, VM74, 25. 6. 1988, A. & M. Gogala leg.

Vinje pri Moravčah, VM71, 23. 5. 1997, A. Gogala leg. Breginj, Logje, 630 m, UM72, 12. 6. 1997, S. Brelih leg. Julijske Alpe: Vas na skali, VM03, 11. 6. 1997, S. Brelih leg.

Ljubljana, Lavrica, VL69, 8. 5. 1991, 20. 5. 1991, V. Furlan leg.

Zaplana, Cesarski vrh, VL49, 27. 5. 1991, V. Furlan leg. Mrzlica, 1100 m, WM01, 28. 5. 1991, V. Furlan leg. Podsreda, Trebča Gorca, WM40, 9. 7. 1998, S. Brelih leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg. lg, Kurešček, VL68, 5. 6. 1983, V. Furlan leg. Ljubljana, Barje, VL69, 1. 5. 1985, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 2. 6. 1984, V. Furlan leg.

#### Hyalochilus dolosus Horváth, 1897

A. Gogala, 1991; Gogala & Gogala, 1994 Specimens examined:

Istra: Sočerga, Veli Badin, VL13, 1. 8. 1990, A. & M. Gogala leg.

Podgorski kras: Kozina, Prešnica, VL14, 22. 6. 1991, V. Furlan leg.

#### Lasiosomus enervis (Herrich-Schaeffer, 1835)

Gogala & Gogala, 1989; Péricart, 1998b: Carniola Specimens examined:

Podkum, Medvedov graben, WM00, 10. 5. 1989, 25. 5. 1989, V. Furlan leg.

Čaven: mountain chalet, 1240 m, VL18, 27. 5. 1999, S. Brelih leg.

Polhograjsko hrib.: Topol, Osredek, VM50, 3. 6. 1984, V. Furlan leg.

#### Stygnocoris cimbricus (Gredler, 1870)

? Gogala & Moder, 1960: Šentvid nad Ljubljano (as *S. pygmaeus*); Gogala & Gogala, 1986, 1989 (as *S. pygmaeus*)

Specimens examined:

Grosuplje, Polica, VL79, 27. 9. 1980, A. & M. Gogala leg.

Dobrova, VM50, 26. 9. 1981 on *Calluna*, A. & M. Gogala leg.

Radlje ob Dravi, WM16, 22. 8. 1984 on *Calluna*, A. & M. Gogala leg.

Sp. Brnik, VM61, 7. 9. 1988, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 25. 8. 2005 on *Calluna vulgaris*, A. Gogala leg.

Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris*, A. Gogala leg. (Fig. 2)

Note: All checked specimens labelled as *S. pygmaeus* proved to be *S. cimbricus*, which confirms the conclusions made by Labina (2003). The true *S. pygmaeus* was synonymized by her with *S. sabulosus*.

#### Stygnocoris fuligineus (Geoffroy, 1785)

Specimens examined:

Ljubljansko barje: Ig, VL69, 4. 7. 1998, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 21. 4. 1985, V. Furlan leg.

#### Stygnocoris matocqi Péricart, 1993

Specimen examined:

Kraški rob: Črni kal, VL14, 22. 10. 1983, M. Gogala leg. (Fig. 2)

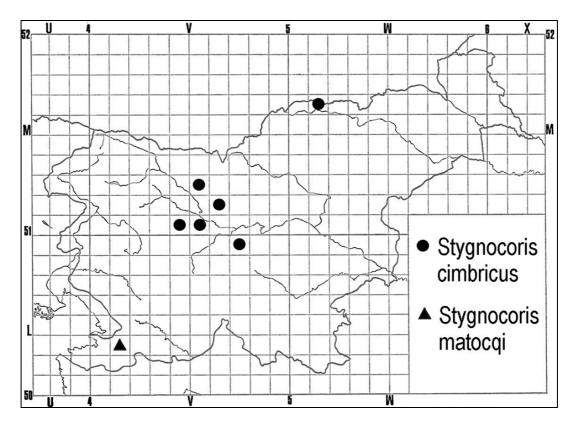


Fig. 2: The distribution of Stygnocoris cimbricus and Stygnocoris matocqi in Slovenia. Sl. 2: Razširjenost vrst Stygnocoris cimbricus in Stygnocoris matocqi v Sloveniji.

#### Stygnocoris rusticus (Fallén, 1807)

Gogala & Moder, 1960: Ljubljana, Dobrova; Gogala & Gogala, 1986, 1989

Specimens examined:

Bohinj: Ukanc, VM02, 10. 9. 1978, A. & M. Gogala leg. Ljubljana: Šiška, VM50, 3. 9. 1980, A. & M. Gogala leg. Istra: Sečovlje, UL93, 20. 9. 1980, A. & M. Gogala leg. Ljubljansko barje: Log, Lukovica, VL59, 21. 9. 1980, A. & M. Gogala leg.

Grosuplje, Polica, VL79, 24. 8. 1982, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 10. 8. 1983, A. & M. Gogala leg.

Radlje ob Dravi, WM16, 22. 8. 1984, A. & M. Gogala leg.

Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 24. 6. 2000, 23. 7. 2000, A. & M. Gogala leg.

Novo mesto, Otočec, WL17, 19. 8. 1996, V. Furlan leg. Muljava, VL88, 24. 8. 1982, V. Furlan leg. Ljubljana, Golovec, VL69, 6. 8. 1982, V. Furlan leg. Zasavje: Polšnik, VM90, 9. 8. 1990, V. Furlan leg.

#### Stygnocoris sabulosus (Schilling, 1829)

Stygnus pedestris (Fallén, 1807)

Stygnocoris pygmaeus (R.F. Sahlberg, 1848)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana, Rožnik, Bohinj, Črni vrh – Novak, Golica; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Bohinj: Ukanc, VM02, 15. 7. 1979, 23. 8. 1980, A. & M. Gogala leg.

Kokra, VM63, 14. 8. 1983, A. & M. Gogala leg. Jezersko, VM64, 14. 8. 1983, A. & M. Gogala leg. Črni kal, VL14, 22. 10. 1983, M. Gogala leg.

Istra: Padna, UL93, 4. 11. 1983, A. & M. Gogala leg. Radlje ob Dravi, WM16, 22. 8. 1984, A. & M. Gogala leg.

Velike Bloke, 2 km E, VL67, 24. 8. 1985, A. & M. Gogala leg.

Bloke: Volčje, Bloško jezero, VL67, 10. 9. 1988, A. & M. Gogala leg.

Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

Brje pri Komnu, VL07, 15. 8. 1990, 10. 10. 1999, 18. 6. 2000, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Kras: Komen, Vale, UL97, 10. 10. 1999, A. & M. Gogala leg.

Polhograjsko hrib.: Črni vrh, VM40, 25. 8. 2005 on *Calluna vulgaris*, A. Gogala leg.

Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris,* A. Gogala leg.

#### **PIESMATIDAE**

#### **Parapiesma quadratum** (Fieber, 1844)

Gogala & Gogala, 1986

Specimens examined:

Istra: Strunjan, UL94, 22. 9. 1982, A. & M. Gogala leg. Sečovlje, Fontanigge, UL93, 6. 5. 2000, A. Gogala leg.

#### Piesma capitatum (Wolff, 1804)

Gogala & Gogala, 1989

Specimens examined:

Istra: Boršt, VL03, 3. 5. 1986, A. & M. Gogala leg. Portorož, UL94, 15. 10. 1986, A. & M. Gogala leg. Kras: Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

#### Piesma maculatum (Laporte, 1833)

Gogala & Moder, 1960: Ljubljana; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Laibach (= Ljubljana), 20. 10. 1928, 20. 9. 1937, 22. 9. 1940, Staudacher leg.

Prekmurje: Lendava, XM15, 6. 7. 1980, A. & M. Gogala leg.

Ljubljansko barje: Log, Lukovica, VL59, 14. 9. 1982, 27. 3. 1983, 17. 4. 1995, 10. 2. 2000, A. & M. Gogala leg. Moravci, WM97, 30. 4. 1983, A. & M. Gogala leg. Kras: Lipica, VL15, 6. 5. 1984, A. & M. Gogala leg. Istra: Boršt, VL03, 3. 5. 1986, A. & M. Gogala leg. Portorož, UL94, 15. 10. 1986, A. & M. Gogala leg. Sečovlje, Fontanigge, UL93, 30. 8. 1989, A. & M. Gogala leg.

gala leg., 20. 6. 2001, A. Gogala leg. Dragonja, Stena, UL93, 9. 6. 1990, A. & M. Gogala leg. Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala

Dobrovnik, Bukovniško jezero, XM07, 23. 5. 1992, A. & M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 14. 5. 2000, 11. 8. 2000, A. Gogala leg.

Škofljica, Grmez, VL69, 27. 2. 2000 in a *Talpa europaea* nest, T. Trilar leg.

Brje pri Komnu, VL07, 21. 10. 2000, A. & M. Gogala leg.

Petišovci, XM15, 10. 4. 1997, S. Brelih leg.

Istra: Kozloviči, VL03, 9. 7. 1997, S. Brelih leg.

Nova Gorica, Panovec, UL98, 15. 5. 2000, S. Brelih leg. Kranj, Brdo, VM52, 31. 8. 2006 on *Calluna vulgaris*, A. Gogala leg.

Gorjanci: Jugorje, WL16, 27. 4. 1983, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. lg, Iška vas, VL68, 3. 5. 1987, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 13. 5. 1991, 21. 5. 1991, V. Furlan leg.

Ljubljana, Barje, VL69, 26. 2. 1992, V. Furlan leg. Ljubljansko barje: Lavrica, VL69, 15. 4. 1991, 23. 4. 1991, 29. 4. 1991, V. Furlan leg.

Kočevje, Slovenska vas, r. Rinža, VL85, 26. 5. 1992, V. Furlan leg.

Ig, Barje, VL69, 4. 7. 1998, V. Furlan leg.

#### **BERYTIDAE**

#### Neides tipularius (Linnaeus, 1758)

Neides favosus Fieber, 1859

Gogala & Moder, 1960: Dol pri Ljubljani, Dobrova, Moravče – Sv. Trojica, Tacen, Novo mesto, Golica, Črnomelj – Tribuče; Gogala & Gogala, 1986 Specimens examined:

Ljubljana, Tacen, VM50, 23. 7. 1931, Staudacher leg. Stadtberg-Rudolfswert (= Novo mesto), WL17, 2. 10. 1932, Staudacher leg.

#### Berytinus clavipes (Fabricius, 1775)

Montandon, 1886: Gorica; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Zagorje ob Savi, WM01, 14. 7. 1935, Staudacher leg. Ljubljansko barje: Ig, VL69, 9. 6. 1940, Staudacher leg. Bärenheim (= Medvedjek, Goteniška gora), VL75, 29. 6. 1936, Staudacher leg.

Istra: Sečovlje, UL93, 20. 9. 1980, 5. 8. 1981, A. & M. Gogala leg.

Slavnik, VL14, 31. 5. 1981, 10. 5. 2000, A. & M. Gogala leg.

Bela krajina: Vinica, Zilje, WL23, 13. 9. 1981, A. & M. Gogala leg.

Medvode, Sora, Draga, VM51, 22. 7. 1982 on *Ononis*, A. & M. Gogala leg.

Jezersko, VM64, 14. 8. 1983, A. & M. Gogala leg. Postojna, Zagon, VL37, 21. 9. 1983, A. & M. Gogala leg.

Pokojišče, VL48, 19. 8. 1984 on *Ononis*, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 2. 5. 1989, 14. 12. 1997, M. Gogala leg., 16. 7. 1989, A. & M. Gogala leg. Bloke: Volčje, VL67, 19. 8. 2005 on *Ononis*, A. Gogala

leg. Istra: Hrvoji, VL03, 21. 7. 1997, S. Brelih leg. Ljubljana, Golovec, VL69, 18. 6. 1982, V. Furlan leg. Ljubljana, Golovec: Orle, VL69, 21. 4. 1985, 4. 5. 1985, 10. 4. 1989, 21. 5. 1991, V. Furlan leg.

Topol, Ravnikar, VM50, 9. 6. 1991, V. Furlan leg. Topol, Osredek, VM50, 12. 5. 1985, V. Furlan leg. Čaven: pl. koča, VL18, 27. 5. 1999, S. Brelih leg. Nova Gorica, Panovec, UL98, 15. 5. 2000, S. Brelih leg.

Muljava, VL88, 27. 5. 1982, V. Furlan leg.

Krma, VM14, 14. 5. 1983, V. Furlan leg.

Radovna, VM24, 14. 5. 1983, V. Furlan leg.

Novo mesto, Trška gora, WL17, 21.-22. 5. 1983, 6. 6. 1987, V. Furlan leg.

Bela krajina: Semič, Gornja Paka, WL15, 29. 4. 1983, V. Furlan leg.

Semič, WL15, 30. 4. 1983, V. Furlan leg. Vinica, WL13, 29. 4. 1983, V. Furlan leg. Podzemelj, WL25, 28. 4. 1983, V. Furlan leg. Bela krajina: Stranska vas, WL15, 28. 4. 1983, V. Furlan leg

Gorjanci: sedlo, 635 m, WL16, 27. 4. 1983, V. Furlan leg.

Polhograjsko hrib.: Grmada, 800 m, VM40, 19. 3. 1983, 6. 5. 1984, V. Furlan leg.

Polhov Gradec, VM40, 6. 5. 1984, V. Furlan leg. Gorjanci: Jugorje, WL16, 27. 4. 1983, V. Furlan leg. Ratitovec, Prtovč, VM22, 10. 6. 1984, V. Furlan leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

Ig, Iška vas, VL68, 3. 5. 1987, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. Podkum, Medvedov graben, WM00, 10. 5. 1989, V. Furlan leg.

Radeče, Jagnjenica, WM00, 24. 5. 1990, V. Furlan leg.

#### Berytinus hirticornis (Brullé, 1836)

A. Gogala *et al.*, 1990; A. Gogala, 1991; Gogala & Gogala, 1994

Specimens examined:

Kras: Brje pri Komnu, VL07, 7. 9. 1989, 29. 7. 1990, 10. 10. 1999, A. & M. Gogala leg.

Istra: Dragonja, Stena, UL93, 17. 9. 1989, A. & M. Gogala leg.

Sočerga, Veli Badin, VL13, 1. 8. 1990, A. & M. Gogala leg.

Podgorje, VL14, 24. 8. 1991, A. & M. Gogala leg. Padna, UL93, 1. 2. 1997, 28. 10. 2000, A. & M. Gogala leg.

Sečovlje, Fontanigge, UL93, 6. 5. 2000, A. Gogala leg.

# Berytinus minor (Herrich-Schaeffer, 1835)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Brežice, Grosuplje; Gogala & Gogala, 1986, 1989 Specimens examined:

Grosslupp (= Grosuplje), VL79, 23. 9. 1933, Staudacher leg.

Ljubljansko barje: Plešivica, VL59, 23. 4. 1978, A. & M. Gogala leg.

Tržič, Visoče, VM43, 1. 5. 1978, A. & M. Gogala leg. Škofja Loka, VM41, 13. 5. 1979, A. & M. Gogala leg. Janče, VM70, 7. 6. 1981, A. & M. Gogala leg. Istra: Koper, Škocjanski zatok, VL04, 18. 5. 1980, A. & M. Gogala leg.

Ig, Kremenica, VL68, 15. 4. 1981, S. Brelih leg. Vrhnika, Log, VL59, 7. 3. 1981, A. & M. Gogala leg. Zg. Radovna, VM14, 28. 8. 1988, A. & M. Gogala leg. Kras: Brje pri Komnu, VL07, 10. 10. 1999, A. & M. Gogala leg.

Dolina Branice: Dolanci, VL17, 13. 2. 2000, A. & M. Gogala leg.

Ljubljana, Lavrica, VL69, 29. 4. 1991, V. Furlan leg.

Ljubljana, Golovec: Orle, VL69, 15. 5. 1983, 20. 5. 1984, 21. 4. 1985, 4. 5. 1985, 10. 4. 1989, 21. 5. 1991, V. Furlan leg.

Ljubljana, Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Želimlje, VL68, 25. 4. 2003, S. Brelih leg.
Ratitovec, 1100 m, VM22, 6. 6. 1982, V. Furlan leg.
Muljava, VL88, 27. 5. 1982, 11. 5. 1985, V. Furlan leg.
Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg.
Radovna, VM24, 14. 5. 1983, V. Furlan leg.
Krma, VM14, 14. 5. 1983, V. Furlan leg.
Polhov Gradec, VM40, 6. 5. 1984, V. Furlan leg.
Topol, Osredek, VM50, 12. 5. 1985, V. Furlan leg.
Bela krajina: Stranska vas, WL15, 28. 4. 1983, V. Furlan

Loški potok, Retje, VL66, 14. 5. 1990, V. Furlan leg.

#### Berytinus crassipes (Herrich-Schaeffer, 1835)

Gogala & Moder, 1960: Zagorje ob Savi; Gogala & Gogala, 1986, 1989

Specimens examined:

Gorjanci: sedlo, 635 m, WL16, 27. 4. 1983, V. Furlan leg.

Ratitovec, 1100 m, VM22, 6. 6. 1982, V. Furlan leg.

#### Berytinus geniculatus (Horváth, 1885)

Gräffe, 1911: Tolmin

Specimen examined:

Kras: Komen, Vale, UL97, 9. 4. 2000, A. & M. Gogala leg.

#### Berytinus montivagus (Meyer-Dür, 1841)

Gräffe, 1911: Tolmin; Gogala & Moder, 1960: Ljubljana, Žiri; Gogala & Gogala, 1989, 1994

Specimens examined:

Laibach (= Ljubljana), 1. 11. 1942, Staudacher leg. Žiri, VM30, 15. 5. 1934, Staudacher leg.

Ljubljana: Savlje, VM60, 15. 4. 1987, A. & M. Gogala leg.

Polhograjsko hrib.: Topol, Osredek, VM50, 12. 5. 1985, V. Furlan leg.

Ljubljansko barje: Vrhnika, Drenov grič, VL49, 18. 2. 1989, M. Gogala leg.

Brje pri Komnu, VL07, 9. 7. 1989, 10. 10. 1999, 13. 2. 2000, 23. 7. 2000, 21. 10. 2000, A. & M. Gogala leg. Ptuj, Pobrežje, WM63, 17. 5. 1990, S. Brelih leg.

Istra: Sočerga, Veli Badin, VL13, 3. 10. 1990, A. & M. Gogala leg.

Ljubljana, Beričevo, VM70, 9. 11. 1996, A. & M. Gogala leg.

Kras: Gorjansko, UL97, 1. 5. 2000, A. & M. Gogala leg. Ljubljana, Golovec: Orle, VL69, 20. 5. 1984, 21. 5. 1991, V. Furlan leg.

Ljubljana, Črnuče, Jarški prod, VM60, 22. 5. 1991, V. Furlan leg.

Topol, Sv. Katarina, VM50, 15. 6. 1997, V. Furlan leg.

Topol, Osredek, VM50, 12. 5. 1985, V. Furlan leg. Topol, Belo, VM40, 5. 5. 1990, V. Furlan leg. Bela krajina: Podzemelj, WL25, 28. 4. 1983, V. Furlan leg.

Povir, VL16, 16. 5. 1984, V. Furlan leg. Mali Kum, 813 m, WM00, 6. 5. 1988, V. Furlan leg. Borovak pri Podkumu, WM00, 30. 4. 1990, V. Furlan leg.

#### Berytinus signoreti (Fieber, 1859)

Horváth, 1887: Gorica; Gogala & Moder, 1960: Šmartno ob Savi, Moravče – Sv. Trojica; Péricart, 1984: Škofja Loka; Gogala & Gogala, 1986, 1989, 1994 Specimens examined:

Podgorski kras: Petrinje, VL14, 20. 9. 1980, A. & M. Gogala leg.

Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

Košuta: Pl. Šija, 1530 – 1800 m, VM44, 20. 8. 1991, A. & M. Gogala leg.

Bloke: Godičevo, VL67, 2. 7. 2000, A. Gogala leg. Kras: Povir, VL16, 16. 5. 1984, V. Furlan leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

#### Berytinus striola (Ferrari, 1874) (Fig. 3)

Péricart, 2001b

Specimens examined:

Istra: Dragonja, Stena, UL93, 6. 5. 2000, A. & M. Gogala leg.

Kras: Brje pri Komnu, VL07, 27. 5. 2000, A. & M. Gogala leg., 19. 11. 2005, M. Gogala leg.

#### Gampsocoris culicinus Seidenstücker, 1948

Gogala & Gogala, 1986, 1994; A. Gogala, 1992; Protić, 2001: Gorica, N. Kormilev leg.

Specimens examined:

Pokojišče, VL48, 7. 9. 1981, A. & M. Gogala leg. Kum, WM00, 26. 7. 1996, A. & M. Gogala leg. Krško, Anovec, WL49, 1. 8. 1996, A. & M. Gogala leg. Hrastnik, WM01, 5. 7. 1997, A. Kapla leg. Kras: Trstelj, UL98, 25. 6. 2000, A. & M. Gogala leg. Sočerga, Mlini, Veli Badin, VL13, 16. 5. 1990, V. Furlan leg.

#### Gampsocoris punctipes (Germar, 1822)

Gogala & Moder, 1960: Brežice

#### Metatropis rufescens (Herrich-Schaeffer, 1835)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Ljubljana, Šklendrovec; Gogala & Gogala, 1986, 1989 Specimens examined:

Laibach (= Ljubljana), 21. 6. 1944, Staudacher leg. Šklendrovec, WM00, 18. 9. 1932, Staudacher leg. Ljubljana: gostilna Pri Čadu, VM60, 29. 10. 1982 on *Rubus*, A. & M. Gogala leg.

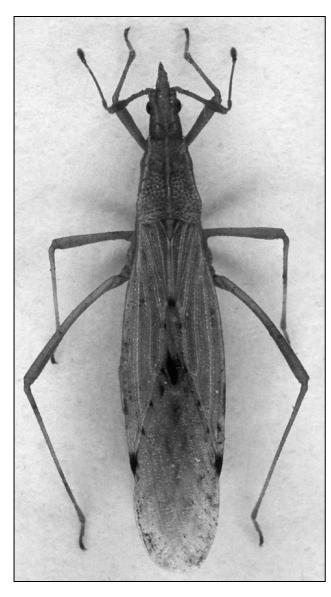


Fig. 3: Berytinus striola (Ferrari) from Dragonja. Sl. 3: Berytinus striola (Ferrari) iz Dragonje.

Makole, Domišaki, WM52, 8. 9. 1983 Mozirje, Nazarje, VM93, 13. 9. 1987, D. Devetak leg. Šmarje pri Jelšah, WM42, 17. 8. 1988, A. & M. Gogala leg.

#### **PYRRHOCORIDAE**

#### Pyrrhocoris apterus (Linnaeus, 1758)

Scopoli, 1763: Carniola; Montandon, 1886: Gorica; Gogala & Moder, 1960; Gogala & Gogala, 1986, 1989, 1994

Specimens examined:

Laibach (= Ljubljana), 15. 6. 1944, Staudacher leg. Medvode, Pirniče, VM51, 13. 2. 1977, A. & M. Gogala leg.

Ljubljana: Savlje, VM60, 5. 3. 1977, A. & M. Gogala leg.

Ljubljana: Šiška, VM50, 16. 2. 1980, A. & M. Gogala leg.

Kamnik pod Krimom, Ponikve, VL58, 20. 6. 1982, A. & M. Gogala leg.

Bovec, UM83, 17. 7. 1982, A. & M. Gogala leg. Strunjan, UL94, 22. 9. 1982, A. & M. Gogala leg. Portorož, Lucija, UL94, 2. 7. 1983, A. & M. Gogala leg. Črni kal, VL14, 22. 10. 1983, M. Gogala leg. Log, Lukovica, VL59, 30. 3. 1987, A. & M. Gogala leg. Štorje, VL16, 26. 3. 1989, A. & M. Gogala leg. Kras: Vojščica, UL97, 6. 5. 1989, A. & M. Gogala leg. Izola, Šared, UL94, 11. 5. 1989, M. Gogala leg. Brje pri Komnu, VL07, 16. 7. 1989, A. & M. Gogala leg. Istra: Izvir Rižane, VL14, 18. 2. 1990, A. & M. Gogala leg.

Nova Gorica, Kromberk, UL98, 2. 10. 1990, M. Gogala leg.

Koper, Bertoki, Škocjanski zatok, VL04, 23. 3. 2000, A. Gogala leg.

Komen, Branik, VL07, 27. 5. 1998, S. Brelih leg. Nanos: Šembijska bajta, 800 m, VL27, 14. 7. 1999, S. Brelih leg.

Muljava, VL88, 26. 1. 1974, V. Furlan leg.
Turjak, VL78, 3. 2. 1974, V. Furlan leg.
Maribor: Piramida, WM55, 3. 9. 1977, V. Furlan leg.
Ljubljana, Golovec, VL69, 21. 6. 1975, V. Furlan leg.
Lipica, VL15, 9. 4. 1978, M. Zdešar leg.
Komen, Mali dol, VL07, 5. 1981, B. Drovenik leg.
Tolmin, Podljubinj, VM01, 6. 1982, B. Drovenik leg.
Polhograjsko hrib.: Grmada, 800 m, VM40, 19. 3. 1983,

V. Furlan leg. Bela krajina: Podzemelj, WL25, 28. 4. 1983, V. Furlan

Novo mesto, Trška gora, WL17, 21.-22. 5. 1983, V. Furlan leg.

Podgorje, VL14, 28. 5. 1983, V. Furlan leg. Rovišče pod Zasavsko Sv. Goro, VM90, 28. 4. 1984, V. Furlan leg.

Povir, VL16, 16. 5. 1984, V. Furlan leg. Topol, Osredek, VM50, 30. 3. 1985, V. Furlan leg.

#### Pyrrhocoris marginatus (Kolenati, 1845)

Montandon, 1886: Gorica; Gogala & Moder, 1960: Dobrova; A. Gogala, 1996 Specimen examined:

Prekmurje: Gančani, WM96, 12. 6. 1993, S. Gomboc leg.

#### Species omitted from the list

#### Aradus annulicornis Fabricius, 1803

Gogala & Moder, 1960: Ljubljana. The record refers to *Aradus corticalis*.

#### Nysius thymi (Wolff, 1804)

Gogala & Gogala, 1986, 1989, 1994. Misidentification, records refer to *N. graminicola*.

#### Kleidocerys ericae (Horváth, 1908)

Ischnorhynchus geminatus Fieber, 1861

Horváth, 1887: Gorica, on *Populus*: dubious record – probably confused with *K. resedae*. The foodplant mentioned actually proves such a conclusion. *K. ericae* lives only in *Erica* and *Calluna* (Péricart, 1998a).

Protić, 1987: Podčetrtek, E. Jaeger leg.: misidentification, the re-examined specimen proved to be *K. resedae* (L. Protić, *pers. comm.*).

#### Geocoris lineola (Rambur, 1839)

Gogala & Moder, 1960: Lendava; Gogala & Gogala, 1986. Misidentification, the record refers to *G. ater*.

#### Megalonotus colon Puton, 1874

Protić, 1987: Podčetrtek, E. Jaeger leg. Misidentification. Ljiljana Protić re-examined the specimen in the Kormilev collection and it turned out to be a *M. hirsutus* specimen with only three antennal segments. This was the cause of misidentification by N. Kormilev (*pers. comm.*).

#### Plinthisus convexus Fieber, 1864

A. Gogala, 1991. Misidentification, records refer to *P. brevipennis*.

#### Raglius pineti (Herrich-Schaeffer, 1835)

? Montandon, 1886: Gorica. Dubious record – probably confused with *R. confusus*.

## Raglius tristis (Fieber, 1861)

Péricart, 1998c: Plössl. Attributed to Slovenia purely by mistake. Ernst Heiss (*pers. comm.*) checked the data written on the label of the specimen in his collection: Yugoslavia, Istrien, Rovinj, 20 VI 76 Plössl leg. Rovinj is in Croatia to which the record refers.

#### Parapiesma kochiae (Becker, 1867)

? Heiss & Péricart, 2001: Slovenia. The published source of this record has not been found. Ernst Heiss checked his collection (*pers. comm.*), but did not find the material from Slovenia. A record for Slovakia was probably mistakenly attributed to Slovenia.

#### **DISCUSSION**

A total of 154 species have been reported for Slovenia, i.e. 14 species of the family Aradidae, 123 of Lygaeidae, 3 Piesmatidae, 12 Berytidae and 2 Pyrrhocoridae. Six species or subspecies have been reported for Slovenia for the very first time: Aradus pallescens frigidus Kiritshenko, 1913, Ischnocoris hemipterus (Schilling, 1829), Stygnocoris cimbricus (Gredler, 1870), Stygnocoris fuligineus (Geoffroy, 1785), Stygnocoris matocgi Péricart, 1993 and Rhyparochromus sanguineus (Douglas & Scott, 1868). The last one, however, is in my opinion only a synonym for Rh. phoeniceus. 9 species, previously reported for Slovenia, have been omitted from the list due to misidentifications or misinterpreted localities. Some other species, reported only once in the literature, also need confirmation. One such species is Gampsocoris punctipes. All Gampsocoris specimens from Slovenia, available to me for examination, are G. culicinus. Changes in the fauna composition, however, are evident. One species, Neides tipularius, was quite common before 1960 according to Gogala & Moder (1960). It has not been found after that year and is presumably extinct or very rare. The cause of its decline is unknown. Warmer climate of the last years probably enabled spread of some species, such as Berytinus striola. The spread across Europe of Arocatus longiceps and Oxycarenus lavaterae is well known. It was detected also in Slovenia, where these species have spread from coastal to the central region in the last few years. Species bound to endangered habitats, however, are endangered themselves. Some of them live only in a few salty marshes along the coast, which are endangered due to ever increasing human pressures. Lamprodema maura, for example, was found at the beach near Ankaran, where the new port facilities are to be constructed. And even in the Sečovlje salina, protected as a nature park, mowing of the reeds tightens the habitat for Dimorphopterus blissoides. The absence of some species from Slovenia is notable. Such species, present in the neighbouring countries but not detected in Slovenia, are Lygaeus simulans Deckert, 1985, Nysius thymi (Wolff, 1804) and Nysius ericae (Schilling, 1829). They are expected to be found in less investigated areas.

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## HETEROPTERA SLOVENIJE, IV: PENTATOMOMORPHA I

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#### **POVZETEK**

Naštete so vrste naddružin Aradoidea, Lygaeoidea in Pyrrhocoroidea, ki živijo v Sloveniji. Navedeni so podatki o pregledanih primerkih. Šest vrst ali podvrst je prvič zabeleženih v slovenski favni: Aradus pallescens frigidus Kiritshenko, 1913, Ischnocoris hemipterus (Schilling, 1829), Stygnocoris cimbricus (Gredler, 1870), Stygnocoris fuligineus (Geoffroy, 1785), Stygnocoris matocqi Péricart, 1993 in Rhyparochromus sanguineus (Douglas & Scott, 1868). Zadnja je sicer po avtorjevem mnenju le sinonim vrste Rh. phoeniceus. 9 vrst je umaknjenih s seznama zaradi napačnih določitev ali napačno razumljenih lokalitet v predhodnih objavah.

Ključne besede: Heteroptera, Pentatomomorpha, Slovenija, favna

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# INTESTINAL CONTENTS OF ADULT *OSMYLUS FULVICEPHALUS* (SCOP.)

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#### **ABSTRACT**

Crop and gut contents of field-collected adults of the European neuropteran species, Osmylus fulvicephalus (Scop.), were examined with glass slide preparations. Well chewed and partially digested insect fragments as well as pollen and fungal spores were found. Aphids, Heteroptera, Coleoptera, Diptera, and mites were noted. We assume that lepidopteran scales, pollen grains and fungal spores were swallowed by osmylid adults while feeding on honeydew. In one female's gut, a well preserved water flea, Chydorus sphaericus, was detected.

Key words: Neuroptera, intestinal contents, pollen, fungal spores, insects, Chydorus sphaericus

# CONTENUTI INTESTINALI DI ADULTI DI *OSMYLUS FULVICEPHALUS* (SCOP.) (NEUROPTERA, OSMYLIDAE)

## SINTESI

Con l'ausilio di preparati microscopici gli autori hanno esaminato i contenuti del gozzo e dell'intestino di individui adulti di una specie europea di neurotteri, Osmylus fulvicephalus (Scop.). Tra i frammenti ben masticati e parzialmente digeriti hanno trovato polline, spore fungali e frammenti di insetti, quali afidi, eterotteri, coleotteri e ditteri. Gli autori suppongono che il neurottero abbia ingerito squame di lepidotteri, polline e spore mentre si nutriva di melata. Nel contenuto intestinale di una femmina è stato ritrovato un esemplare ben conservato di una specie di cladocero, Chydorus sphaericus.

Parole chiave: Neuroptera, contenuti intestinali, polline, spore fungali, insetti, Chydorus sphaericus

#### **INTRODUCTION**

The feeding biology of some lacewings (Neuroptera) is still insufficiently known. Adults and larvae of most neuropteran species are free-living predators and generalist feeders as they take a wide variety of small, soft bodied arthropod prey (Principi & Canard, 1984; Stelzl, 1991; Stelzl & Devetak, 1999). According to their feeding habits, some neuropteran families have been recognised as important predators in agroecosystems (e.g. Stelzl & Gepp, 1987; New, 1989; Stelzl, 1990; Stelzl & Devetak, 1999; Duelli, 2001; McEwen et al., 2001). Only a few exceptions exist among lacewings, feeding on food of plant origin. Adults of Sisyridae and Osmylidae take both animal and plant food, and adults of many Chrysopidae are pollen or honeydew feeders (Tjeder, 1944; Kokubu & Duelli, 1983, 1986; Canard et al., 1984; New, 1989; Stelzl & Devetak, 1999; McEwen et al., 2001). On the other hand, adults of Nemopteridae seem to feed exclusively on pollen (Tjeder, 1967; Monserrat, 1996).

Osmylus fulvicephalus (Scopoli, 1763), the only wide-spread European osmylid species, occurs near water bodies, along streams and river banks. David (1936) reported that the species feeds on flowers, dead insects of various orders, and attacks conspecifics as well. Killington (1932) noted lepidopteran scales in its alimentary tract. Kokubu & Duelli (1986) examined the crop and gut contents of field-collected adult *O. fulvicephalus* and found a variety of food, consisting of fragments of insects, eriophyid mites, pollen, and algae. Stelzl (1991) detected aphids, dipterans and honeydew. The aim of this study is to present results of the observations on intestinal contents of *O. fulvicephalus* collected in central and southern Europe.

# **MATERIAL AND METHODS**

Osmylus fulvicephalus adults were collected using a sweepnet and preserved in 70% ethanol. Individuals from the following places were used in the study: Croatia: Buzet, Bračana, 21. 5. 1986, 1 male, leg. C. Krušnik; Italy: Campigna National Park, Gran Duca Hotel, 25. 6. 2005, 2 males, leg. D. Devetak; Macedonia: Pletvar, Prilep, 28. 5. 1979, 1 male, leg. P. Jakšić; Montenegro: Durmitor National Park, G. Dobrilovina, 15. 6. 1985, 3 males, 2 females leg. D. Devetak and F. Janžekovič; Slovenia: Maribor, Zg. Radvanje, 22. 6. 1986, 3 males, 3 females, leg. D. Devetak; Slovenia: Medvode, Trnovec in the Ločnica valley, 24. 6. 1976, 2 females, leg. D. Devetak; Slovenia: Slovenj Gradec, Bukovska vas, 22. 5. 1983, 2 males, 2 females, leg. L. Slana, M. Ferenc, M. Stangelj, T. Novak; Slovenia: Tacen, 19. 6. 1976, 1 female, leg. D. Devetak.

Twenty-two individuals (13 males, 9 females) were dissected and parts of their digestive tract with the con-

tents were mounted in 70% ethanol on glass slides and examined microscopically. Identification of the arthropod fragments required extensive studies of literature on insect morphology (e.g. Meijere, 1901; Borror et al., 1976; Steinmann & Zombori, 1985; Dettner & Peters, 1999 etc.) and preparation of glass slides for comparative analysis.

#### **RESULTS**

As the crop and gut contents showed no basic difference, and as there was no difference detected between the sexes, results on crop and gut contents of both sexes are presented together.

The crop and gut contents of almost all specimens contained well chewed insect fragments (Tab. 1). The exceptions were one male from Buzet (Croatia) and two freshly emerged males collected in Campigna National Park (Italy), which were obviously caught before feeding, for their crops and guts were empty. Only in a few cases it was possible to identify the insect order for the corresponding fragment, but mostly the origin of arthropod remains was unknown. In the preparations, fragments of insect antennae, compound eyes, legs, and wings were noted. Compound eyes frequently occurred in the intestinal contents, differing in the shape of lenses of their ommatidia (Figs. 1-2). Fragments of eyes with hexagonal lenses were common (Fig. 1) whereas those with heteropteran-like lenses were noted only twice (Fig. 2). Dipteran, coleopteran and heteropteran tarsi and lepidopteran scales were also found (Figs. 4-6). Aphids were present in the crops and guts of numerous preparations; in one female, an almost complete aphid was documented

Tab. 1: Occurrence of food items in crop and gut contents of O. fulvicephalus in terms of absolute numbers. Legend: N = total number of individuals investigated microscopically; n = number of individuals containing certain food item.

Tab. 1: Pojavljanje ostankov hrane v prebavilih vrste O. fulvicephalus, izraženo v absolutnih številkah. Legenda: N = število vseh pregledanih osebkov; n = število osebkov z ostanki določene hrane.

| Food source (N = 22)             | n  |
|----------------------------------|----|
| Homoptera: aphids                | 7  |
| Heteroptera                      | 2  |
| Coleoptera                       | 4  |
| Diptera                          | 5  |
| Lepidoptera (scales)             | 5  |
| Acarina                          | 1  |
| Crustacea: Cladocera             | 1  |
| pollen                           | 9  |
| Fungi: micelia, spores           | 6  |
| unidentified arthropod fragments | 19 |

(Fig. 3). In another individual, a partially fragmented mite was recorded.

Unexpected was the finding of a well preserved water flea, *Chydorus sphaericus* (Fig. 7). This cladoceran was found in a female originating from a place close to a pond (Zg. Radvanje, Slovenia).

In a majority of digestive tracts, different kinds of pollen were found. Some of them were determined as pollen of the families Pinaceae (*Picea abies, Pinus*) and Apiaceae (Figs. 10-12). Mycelia (Fig. 8) and spores of unidentified fungi were common. In a few individuals (originating from Slovenia and Montenegro), asexual spores (conidia), called phragmospores, of unidentified fungi (probably of the Ascomycota-group) were recorded (Fig. 9).

#### **DISCUSSION**

The majority of Neuroptera are known as predators, feeding on various soft bodied arthropods. Based on arthropod fragments dominating in crops and guts, one can conclude that *Osmylus fulvicephalus* is mainly carnivorous. For both neuropteran families, Osmylidae and Sisyridae, algae, fungi and foliage were found to be an additional diet (Kokubu & Duelli, 1983, 1986). Fungal spores, fragments of heteropterans, coleopterans and cladocerans had not yet been documented for *O. fulvicephalus* before (Tab. 2). In neuropterid families, only the Nemopteridae seem to feed exclusively on pollen, and are thus considered trophic specialists (Monserrat, 1996).

In the gut of dissected specimens of Australian Kempyninae, a subfamily of Osmylidae, New (1983) found

pollen, fungi, and fragments of bark and foliage, but there was a lack of food of animal origin (Tab. 2).

Sheldon & MacLeod (1971) noted that adult green lacewings of species with non-predatory adults in the genus *Chrysoperla* scrape at the leaf and twig surface where honeydew is present. For the same species it was confirmed that sooty molds (Dematiaceae) growing directly on the honeydew are ingested with it (Sheldon & MacLeod, 1971). Lepidopteran scales can get trapped in the sticky honeydew when lepidopterans (butterflies and moths) fly close to homopterans producing honeydew. So the presence of lepidopteran scales in digestive tract does not necessarily mean that butterflies or moths are consumed by predators.

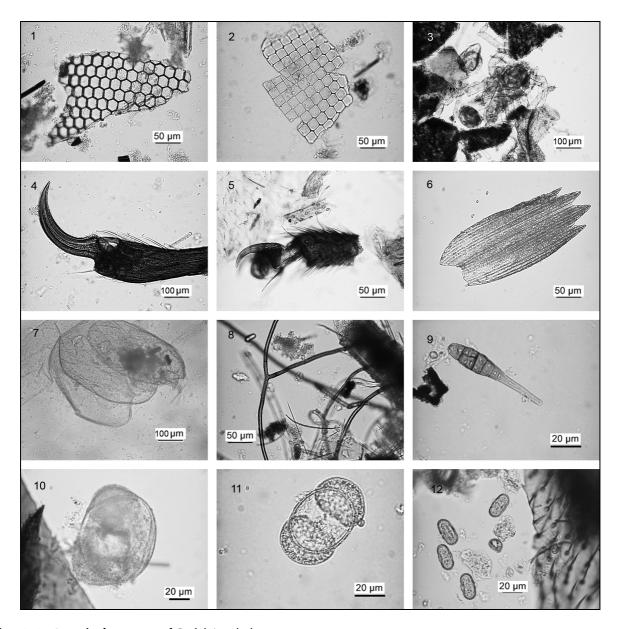
David (1936) reported that osmylid adults occasionally feed on dead insects and may even prey on weak conspecifics. The majority of individuals inspected in the present study contained also pollen, some of them in large amounts. As already suggested by Kokubu & Duelli (1986), the species does seem to actively feed on pollen. Obviously, some pollen grains and fungal spores could also have been swallowed by osmylid adults while feeding on honeydew.

In one female's gut, a water flea (Chydorus sphaericus; Cladocera, Crustacea) was recorded. We assume that the flea was swallowed accidentally, probably while feeding on honeydew from the surface of floating leaves of water plants, which are often covered with aphids. A splash of a frog or a heavy raindrop may have catapulted the flea onto the leaf. It is known that water fleas at times occur in masses at the water surface. The speculation on the occasional acquisition of the cladoceran is supported by the fact that it was found almost intact, as

Tab. 2: Intestinal contents of Osmylinae and Kempyninae. Tab. 2: Vsebina prebavil poddružin Osmylinae in Kempyninae.

|                      | Subf      |            |           |
|----------------------|-----------|------------|-----------|
| Food source          | Osmylinae | Kempyninae | Reference |
| Homoptera: aphids    | X         |            | 3, 4, 5   |
| Homoptera: psyllids  |           | Х          | 2         |
| Heteroptera          | X         |            | 5         |
| Coleoptera           | X         |            | 5         |
| Diptera              | X         |            | 3, 4, 5   |
| Lepidoptera          | X         | X          | 1, 2, 5   |
| Acarina: Eriophyidae | X         |            | 3         |
| Crustacea: Cladocera | X         |            | 5         |
| pollen               | X         | X          | 2, 3, 5   |
| algae                | X         |            | 3         |
| fungi                | X         | X          | 2, 5      |
| bark fragments       |           | X          | 2         |
| foliage fragments    |           | X          | 2         |
| mineral particles    | X         |            | 3         |

References / Reference: 1 - Killington (1932); 2 - New (1983); 3 - Kokubu & Duelli (1986); 4 - Stelzl (1991); 5 - this paper.



Figs. 1–12: Intestinal contents of O. fulvicephalus.

- Sl. 1–12: Vsebina prebavil mrežekrilca O. fulvicephalus.
- Fig. 1: Fragment of a compound eye with hexagonal lenses. / Sl. 1: Fragment sestavljenih oči s heksagonalnimi lečami.
- Fig. 2: Fragment of a compound eye with quadratic lenses (a heteropteran-like eye). / Sl. 2: Fragment sestavljenih oči s kvadratnimi lečami (struktura spominja na oči stenic).
- Fig. 3: An aphid. / Sl. 3: Listna uš.
- Fig. 4: Distal part of a coleopteran tarsus. / Sl. 4: Distalni tarzalni del hrošča.
- Fig. 5: Fragment of a dipteran tarsus. / Sl. 5: Tarzalni fragment dvokrilca.
- Fig. 6: Lepidopteran scale. / Sl. 6: Luskica metulja.
- Fig. 7: A water flea, Chydorus sphaericus. / Sl. 7: Vodna bolha, Chydorus sphaericus.
- Fig. 8: Septate fungal hyphae. / Sl. 8: Septirane hife gliv.
- Fig. 9: Phragmospore. / Sl. 9: Fragmospora.
- Fig. 10: Pollen grain of spruce fir, Picea abies. / Sl. 10: Pelodno zrno smreke, Picea abies.
- Fig. 11: Pollen grain of pine, Pinus sp. / Sl. 11: Pelodno zrno bora, Pinus sp.
- Fig. 12: Pollen of Umbelliferae. / Sl. 12: Pelod kobulnice.

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it was too small to be masticated into smaller pieces before it was swallowed. Kokubu & Duelli (1986) found in *O. fulvicephalus* green and brown coloured algae. Both findings indicate that adult osmylids are strongly linked to water bodies for feeding.

The size of neuropteran insects and the morphology of their mouthparts reflect the feeding ecology of a taxon. The morphology of the mouthparts in Nemopteridae proves that they are feeding only on pollen (Monserrat, 1996). Sisyridae (with *Sisyra terminalis* as an example) feed mainly on aphids and eriophyid mites. This specialization may be due to their minute size (Kokubu & Duelli, 1983). Larger Neuroptera, like osmylids, ascalaphids, and myrmeleontids (Kokubu & Duelli, 1986; Stelzl & Gepp, 1990; Stelzl, 1991; Devetak *et al.*, 2002) are general predators feeding on a variety of arthropods.

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# VSEBINA PREBAVIL ODRASLEGA MREŽEKRILCA *OSMYLUS FULVICEPHALUS* (SCOP.) (NEUROPTERA, OSMYLIDAE)

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#### **POVZETEK**

S tehniko izdelave mikroskopskih preparatov smo preučevali vsebino golše ter srednjega in zadnjega črevesa evropske vrste potočnega mrežekrilca, Osmylus fulvicephalus (Scop.). Med dobro prežvečenimi in delno prebavljenimi fragmenti smo našli pelodna zrna in spore gliv, med ostanki členonožcev pa listne uši, stenice, hrošče, dvokrilce ter pršice. Domnevamo, da je mrežekrilec pogoltnil luskice metuljev, pelodna zrna in spore gliv, ko se je hranil z medeno roso. V prebavilih enega osebka smo našli dobro ohranjeno vodno bolho vrste Chydorus sphaericus.

Ključne besede: Neuroptera, vsebina prebavil, pelod, spore gliv, žuželke, Chydorus sphaericus

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# BIENNIAL BEARING IN OLIVE (OLEA EUROPAEA)

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#### **ABSTRACT**

Alternate bearing is a wide spread phenomenon in many fruit tree species and causes severe labor, marketing and thus economical problems. The domestic olive (Olea europaea) is genetically highly alternating in fruit production. The expression of alternate bearing in olives involves a wide range of changes in activation and repression of endogenous metabolic pathways. The degree of alternate bearing is highly dependent on the environmental conditions and might be very different in accordance with the climate in each growing region. The objective of this work was to present the main endogenous and environmental factors and their interactions that lead to alternate bearing and to review approaches with which alternate bearing is reduced.

Key words: Olea europaea, physiology, flower bud induction, alternate bearing, biennial bearing reduction

# PRODUTTIVITÀ BIENNALE DELL'OLIVO (OLEA EUROPAEA)

#### SINTESI

La produttività alternata è un fenomeno molto diffuso negli alberi da frutta e causa notevoli problemi lavorativi, di marketing ed economici. L'olivo (Olea europaea) è geneticamente altamente alternante nella produzione di frutti. L'espressione della produttività alternata degli olivi include un'ampia sfera di cambiamenti nell'attivazione e nella repressione delle sequenze del metabolismo endogeno. Il grado di produttività alternata dipende in gran parte dalle condizioni ambientali e può variare a seconda del clima di ogni singola regione di crescita. Lo scopo di tale studio era quello di presentare i principali fattori endogeni ed ambientali e le interazioni fra essi che portano alla produttività alternata. L'articolo fornisce inoltre una revisione degli approcci con i quali la produttività alternata viene ridotta.

**Parole chiave:** Olea europaea, fisiologia, induzione a fiore delle gemme, produttività alternata, riduzione bienniale della produttività

#### **INTRODUCTION**

Biennial or alternate bearing is a widely spread phenomenon in many fruit tree species and causes severe labor, marketing and thus economical problems. Still, the metabolic processes, their induction and the messengers involved are only very partially known. There is no doubt, however, that the processes involved are not universal and rather different in the various fruit tree species (Goldschmidt, 2005). Although different horticultural practices have been developed and are being used to minimize the alternate bearing in many species, their effect is in most cases only partial (Monselise & Goldschmidt, 1982).

The domestic olive (Olea europaea) is genetically highly alternating in fruit production. In non-irrigated olive groves the yield may vary between 7-8 tons/ha and a few hundreds kg. The occurrence and development of alternate bearing is potent also in intensive orchards with controlled irrigation, nutrition and training techniques, though the level of fruit production is higher and better controlled (Lavee, 1989). Without specific intervention, the gap between 'off' and 'on' years may vary between 5-30 t/ha. As the olive is an industry dependent commodity, the problems rising from alternate bearing are of particularly high economical severity. The degree of alternate bearing is highly dependent on the environmental conditions and might be very different in accordance with the climate in each growing region (Morettini, 1950; Hartmann, 1951). The impact of the environmental conditions is not only of direct nature on the reproductive organs - the flowers - but have also a major impact on the endogenous metabolic processes of the tree. This impact involves metabolic changes induced by specific gene activation or repression.

# THE EFFECT OF FRUITING AND VEGETATIVE GROWTH ON ALTERNATE BEARING OF OLIVE TREES

To address this problem, it is necessary to clarify first the fruiting habit of the trees. The olive fruit develops on inflorescences developing from the buds borne on the previous year's shoots. However, inflorescences will develop only on well lignified wood, thus stronger and longer vegetative shoots grown in the previous year have a better potential to develop inflorescences in the present one. Olive shoots grow throughout the summer and in many regions from very early in the spring to late fall and in extreme cases even in the winter. The buds developing in the leaf axils will be therefore at different ages in the following spring at time of bloom. Still, all the buds on well-lignified parts of the shoot reach about the same level of development at the end of the summer and can potentially differentiate to form inflorescences. In extreme cases (years or regions) even the very late growth may be lignified and terminal inflorescences can

be found. The number of flowers per inflorescence varies between 10-32 according to variety and year. The potential number of flowers per tree particularly in 'on' years is extremely high. A varying number of the flowers on each inflorescence are non-producing male flowers. The amount of fruit per tree is not dependent on the percent of male flowers in either the 'on' and 'off' years, as only 1-3 flowers per inflorescence (and not on all of them) set fruit (Tab. 1). The reproductive and vegetative buds are of the same origin. Once its inflorescent developed, a reproductive bud cannot form a vegetative shoot even when it did not set any fruit. At a very high fruiting event even the few lateral buds, induced to develop extension growth and generally vegetative terminal buds, are inhibited and develop rather weak shoots. Under such conditions the amount of shoots, their length and thus the number of buds available for differentiation and fruiting in the following year is very low. The potential of the buds on such shoots to differentiate into reproductive buds is low even under favorable environmental conditions. This is due to endogenous metabolic changes leading to inhibition of flower bud differentiation in general and viable flowers in particular (Cuevas et al., 1994; Kitsaki et al., 1995).

The 'off' year, which will result, is usually characterized by an establishment of vigorous vegetative growth. The relatively long and strong shoots during that year bear a large number of well-developed buds, which under suitable environmental conditions are ready to undergo reproductive differentiation. The large number of buds that potentially could differentiate into reproductive ones is the basis for reestablishing an extensive number of inflorescences, which will usually lead to the development of the next 'on' year.

It could be concluded that fruit production in the olive is mainly dependent on the vegetative growth of the previous growing season. On the other hand, the degree of vegetative growth in any particular season is a function of the amount of fruit present on the tree at the same time. Thus, the balance between the amount of developing fruit and the vegetative growth in any given growing season will effect and control the potential fruit production for the following season.

# THE EFFECT OF FRUIT ON FLOWER BUD INDUCTION

In addition to the effect of fruit on the level of vegetative growth, the developing fruits were shown to have also a significant effect on the development of flower buds for the following season. For various fruit species it was suggested that the developing fruit is a strong sink competing for metabolites with the vegetative growth (Monselise & Goldschmidt, 1982). In olive, this relation is not clear and seems to be not particularly significant. Some workers found a correlation between 'on' and 'off'

Tab. 1: Relationship between the percent of perfect flowers and fruit-set per 100 inflorescences in three olive cultivars.

- A, B, C = groups of shoots with different levels of perfect flowers within each of the three cultivars tested.
- Tab. 1: Razmerje med odstotki popolnih cvetov in nastavkom plodov za 100 socvetij pri treh oljčnih kultivarjih.
- A, B, C = skupine poganjkov z različnimi stopnjami popolnih cvetov znotraj posameznega testiranega kultivarja.

| Inflorescence<br>Group | cv. Sevillano |                | cv. Suri    |                | cv. Mazanillo |                |
|------------------------|---------------|----------------|-------------|----------------|---------------|----------------|
|                        | Perfect       | Fruits/100     | Perfect     | Fruits/100     | Perfect       | Fruits/ 100    |
|                        | flowers (%)   | inflorescences | flowers (%) | inflorescences | flowers (%)   | inflorescences |
| Α                      | 5             | 12             | 25          | 27             | 35            | 72             |
| В                      | 20            | 14             | 45          | 26             | 55            | 69             |
| С                      | 35            | 13             | 65          | 28             | <i>7</i> 5    | 71             |
| MSE                    |               | 4              |             | 6              |               | 6              |

Tab. 2: The protein content of mature olive leaves and one year old bark of four cultivars in 'on' and 'off' years (sampled in late summer, expressed in μg/g f.wt). Different letters represent significance at the P=0.05 level.

Tab. 2: Vsebnost proteinov v odraslih oljčnih listih in lubju enoletnega poganjka v rodnem in nerodnem letu (vzorčeno pozno poleti, izraženo v μg/g svežih listov). Različne črke ponazarjajo statistično značilnost pri P=0,05.

| Tree phase | Manzanillo | Barnea | Uovo  | Koronaiki |  |  |  |  |
|------------|------------|--------|-------|-----------|--|--|--|--|
|            | Leaves     |        |       |           |  |  |  |  |
| 'off'      | 295 a      | 310 a  | 370 a | 475 bc    |  |  |  |  |
| 'on'       | 510 b      | 405 c  | 510 b | 530b      |  |  |  |  |
| Bark       |            |        |       |           |  |  |  |  |
| 'off'      | 440 b      | 500 b  | 490 b | 490 b     |  |  |  |  |
| 'on'       | 360 a      | 370 a  | 360 a | 370 a     |  |  |  |  |

years with primer metabolites, such as carbohydrates (Seyyednejad et al., 2001) and polyamines (Pritsa & Voyiatzis, 2004). An increase in the starch content during the winter in the central axis of lateral potentially reproductive buds was also reported (De la Rosa et al., 2000). Others, however, did not find a competitive effect for basic organic metabolites between the developing fruiting and vegetative growth (Fernandez-Escobar et al., 1999, 2004). Stutte & Martin (1986a) found a uniform level of carbohydrates in the leaves of bearing and non bearing trees. They also found that destruction of the seeds (embryos) in intact fruits before stone hardening allowed a seedless development of the fruits. Such "partenocarpic" fruits had only a small effect on the returned bloom and crop development in the following year (Stutte & Martin, 1986b). It could be concluded that the effect of the developing fruits on reducing flower bud differentiation for the following season is of regulatory nature via signals produced by the developing embryos. Such signals could be involved in developing the significant differences found in the protein content and its quantitative changes during the growing season in both leaves and young shoots of fruiting and non-fruiting trees (Lavee & Avidan, 1994). Seasonal change in the amount of total proteins in the leaves and young shoots found during 'on' and 'off' years revealed opposite trends (Tab. 2). These differences in the protein content

seem to be more of regulatory nature, as specific and different proteins were induced during the 'on' years and others in the 'off' ones. The nature of these proteins and their possible role in controlling alternate bearing are presently investigated at the Volcani Center in Israel.

Lately, specific changes in the mineral content of leaves between 'on' and 'off' years were reported and related mainly to the potential activity of growth regulating systems (Fernandez-Escobar et al., 1999, 2004). Troncoso et al. (2006) showed a considerable depletion of the N and K contents in the leaves at the end of the 'on' year, while at the end of the 'off' year these values were high (Tab. 3). They concluded that a recovery of the mineral content is required for flower bud differentiation to reoccur. Growth regulators and particularly gibberellins were shown to reduce flower bud induction in the olive as in many other fruit species when applied during the major growing season in the summer or in the fall (Lavee, 1989; Fernandez-Escobar et al., 1992; Lavee & Haskal, 1993). In an old paper, however, Badr et al. (1970) showed that at least two gibberellins increased during the winter in lateral expected flower buds, while abscisic acid was higher in the terminal vegetative buds. Differences in the content of various growth regulators in leaves and buds during 'on' and 'off' years were reported by various workers (Navaro, 1990; Ben-Tal & Wodner, 1993; Baktir et al., 2004). The major changes

Tab. 3: Leave-nutrient contents in different phases of the production cycle.

Tab. 3: Vsebnost hranil v listih v različnih obdobjih rodnega cikla.

| Biannual cycle phase             |  | % d.w.      |      |      |      | ppm  |    |    |    |
|----------------------------------|--|-------------|------|------|------|------|----|----|----|
| Biaimuai Cycle phas              | se   | N P K Ca Mg |      | Mg   | Fe   | Mn   | В  |    |    |
| Winter stop afte                 | r harvest  | 1.45        | 0.07 | 0.50 | 2.10 | 0.14 | 70 | 27 | 12 |
| Recovery after                   | Spring   | 1.67        | 0.10 | 0.60 | 1.70 | 0.15 | 40 | 24 | 13 |
| harvest                          | Summer   | 1.65        | 0.12 | 1.00 | 1.20 | 0.10 | 35 | 22 | 25 |
| ('off' year)                     | Autumn   | 1.67        | 0.13 | 0.90 | 1.20 | 0.13 | 50 | 20 | 18 |
| Winter stop before harvest       |  | 1.75        | 0.12 | 0.80 | 1.30 | 0.13 | 50 | 20 | 14 |
| Emilt dayalanmant                | Spring   | 1.64        | 0.09 | 0.60 | 1.30 | 0.12 | 55 | 23 | 14 |
| Fruit development<br>('on' year) | Summer   | 1.56        | 0.08 | 0.50 | 1.60 | 0.13 | 65 | 30 | 20 |
|                                  | Autumn   | 1.40        | 0.06 | 0.40 | 2.20 | 0.14 | 70 | 30 | 14 |
| Optimal Nutritio                 | Optimal Nutritional Level         1.95         0.11         0.86         1.42         0.20         39         50 |             | 50   | 15   |      |      |    |    |    |

Tab. 4: The effect of winter and spring applications of exogenous chlorogenic acid (CHA) on flower bud differentiation and fruit-set on cv. Manzanillo trees. (CHA was pressure injected in scaffolds.)

Tab. 4: Vpliv zimskega in pomladnega tretiranja s klorogensko kislino na diferenciacijo cvetnih brstov in nastavek plodov pri drevesih cv. Manzanillo. (CHA je bila dodana s škropljenjem.)

| Treatment of branch | Inflorescences               |     | Fruit set (%) | Fruit      | lets         |  |
|---------------------|------------------------------|-----|---------------|------------|--------------|--|
|                     | No./branch % of control      |     |               | No./branch | % of control |  |
|                     | 4 injections 10 Dec – 10 Feb |     |               |            |              |  |
| Untreated           | 227                          | 100 | 26            | 59         | 100          |  |
| CHA injected        | 118                          | 52  | 23            | 27         | 46           |  |
|                     | 3 injections 15 Feb – 25 Mar |     |               |            |              |  |
| Untreated           | 220                          | 100 | 28            | 62         | 100          |  |
| CHA injected        | 215                          | 98  | 30            | 65         | 105          |  |
| MSE                 | 10                           | _   | _             | 4          | _            |  |

in the various growth regulators content and the ratio between them were found during specific developmental periods. These periods could be related to bud differentiation, such as mid summer, early and late winter and before bud opening in the early spring (Bakir *et al.*, 2004). The involvement of growth regulators and the balance between them regulates the current vegetative and fruit development but at the same time act as vectors to initiate the specific metabolic activity controlling the fruiting potential for the following year.

A significant change in the content of secondary metabolites, such as chlorogenic acid in the leaves of olive trees between 'on' and 'off' years, was noted. It is assumed that the signal inducing the synthesis and accumulation of these phenolic metabolites in the leaves is initiated in the developing embryos in the fruits. This could be partially verified by removing the young developing fruit-lets and preventing there by the increase in the level of chlorogenic acid in the leaves, keeping it at a similar one as found in the leaves on 'off' year trees (Lavee & Avidan, 1981; Lavee et al., 1986; Ryan, et al., 2003). Injection of chlorogenic acid during the winter before an 'on' year into the xylem of trees in the field reduced flower bud differentiation on the treated scaffolds by more than 50% (Tab. 4). A similar application

in the early spring before bud opening had no effect on bud differentiation and inflorescences development (Lavee *et al.*, 1986). A rather high positive correlation was found in mid summer between the amount of fruit per tree and the level of chlorogenic acid in its leaves. It could be concluded therefore that the developing fruits in the present year are not only in competition with the vegetative growth but have also a direct effect on the metabolism leading to reproductive induction and differentiation of the buds for the potential yield in the following year (Fig. 1).

Generally, the negative correlation between the amount of yield in the present year and that in the following one was already well established in the past as the basis for alternate bearing, as was also the effect of late harvesting in the second half of the winter. In the case of late harvesting it might be speculated that an additional independent effect on bud growth inhibition can be active on top of the earlier induced effect of yield on bud differentiation leading to the reduced flowering and some times fruit set in the following spring. The quality and viability of flowers developing in the year following a heavy yield was considerably reduced (Cuevas *et al.*, 1994).

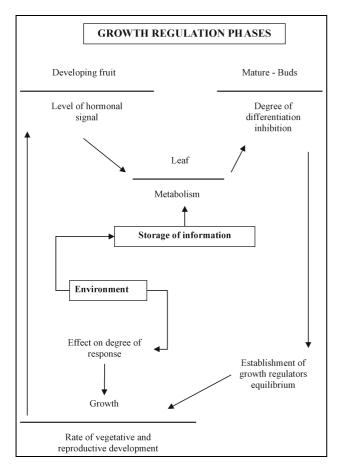


Fig. 1: A flow diagram of the stages involving growth regulators in controlling the level of alternate bearing in olive trees.

Fig. 1: Prikaz stopenj, ki vključujejo regulatorje rasti v procesu alternativne rodnosti oljčnih dreves.

#### EFFECT OF CLIMATE ON ALTERNATE BEARING DEVELOPMENT AND EXPRESSION IN OLIVE ORCHARDS

Under normal environmental conditions suitable for olive development, alternate bearing develops gradually and on an individual tree basis. At a young age and particularly under good growing and intensive conditions yield is increasing gradually during the first 3-4 fruiting years and alternate bearing usually does not develop. Thereafter, olive trees will gradually start to alternate in their production, unless specific horticultural practices such as adequate pruning, thinning etc. will be applied. The alternate bearing will be under annual favorable climatic conditions very light and often even unnoticed for the whole orchard or a region. Under such conditions it is independent of the climate and develops individually for each tree based on the balance between its previous individual growth and fruit production. As a result, the mean production of the orchard could be rather

uniform with only slight fluctuations for many years, particularly when adequate horticultural care is given to the trees. This will not be the case in regions where the climate is unstable and might in some years be limiting and in others particularly favorable for flower bud differentiation and fruit set. Such conditions occur mainly in the warmer growing regions where winter temperatures (particularly the schedule and amount of chilling) vary significantly from year to year. Regions with occasional spring frost, heavy rains or dry and hot desert winds during flowering will also initiate the onset of a sudden orchard or regional biannual bearing. The gradual development of alternate bearing of individual trees is controlled by their endogenous metabolism, while the sudden synchronized beginning of orchard or regional biannual bearing is primarily induced by external environmental factors.

Temperature is the major environmental factor influencing the process leading to flower differentiation. The requirement of adequate amounts of winter chilling (below 9 °C) for flower bud differentiation was suggested many years ago (Morettini, 1950; Hartmann, 1951). Unfavorable temperature regimes particularly in the winter are instrumental in inducing alternate bearing in general and orchard or regional synchronized one in particular. In regions with uniformly cool winter temperatures, the potential for flower buds to differentiate in the trees is high. Small differences in vegetative growth or fruit production between years will gradually lead to alternate bearing development with an individual schedule for each tree. In regions with relatively warm winters and marginal chilling hours, years with somewhat increased chilling conditions will induce an abundant amount of reproductive buds in all trees. This potentially leads to a high uniform fruit production that will result in an overall low flower bud differentiation for the crop of the following year and there cause the beginning of a synchronized alternate bearing. More common though in inducing synchronized alternate bearing will be the opposite situation, starting with an 'off' year caused, i.e. by lack of enough suitable chilling conditions (Hartmann & Prolingis, 1957). The climatic conditions have a major impact on olive tree fruiting and development at different stages during its annual developmental cycle. In non-irrigated trees, the amount of rain and its distribution is governing the level of tree development and its potential fruit production together with the thermal schedule. Any extreme condition leading to a water or thermal stress at any stage during the growth cycle of the tree may induce a misbalance between vegetative development and fruiting, which could result in an initiation of alternate bearing. Even a single and short stress incident when occurring at a critical developmental stage can create such a misbalance and start a biannual bearing syndrome. A most severe initiation of alternate bearing will occur in both non-irrigated and ir-

rigated orchards when flowering or fruit set are climatically damaged. High temperatures and dry winds on the one hand and heavy rain or frost on the other might lead to a fruitless, highly vegetative season, which in turn will result in over-cropping during the following year. The resulting biannual bearing might persist for many years, if horticultural means to minimize it are not taken. In irrigated orchards, the major climatic parameter effecting fruit production is the temperature as water stress can and usually is avoided by the controlled water supply. It is commonly assumed that biannual bearing in intensive orchards is less severe; this, however, is usually not the case. Furthermore, in many regions it is even more extreme due to the vigorous vegetative growth in the 'off' years and thus followed by an extensive fruit production in the 'on' ones creating a vicious cycle of extreme alternate bearing.

Temperature conditions are closely involved in the processes leading to flower bud differentiation and viability of the flowers. In warm growing regions, winters with insufficient chilling for normal flower bud development are rather frequent. The nature of the low temperature effect on reproductive bud development is not yet resolved. In various studies, different interpretations were suggested for the effect of temperature and particularly the low ones during winter on the reproductive development of the olive. Some workers suggested that the winter chilling is required to release pre-determined flower buds from a dormant phase enabling the inflorescences development for a forthcoming yield (Rallo & Martin, 1991; Rallo et al., 1994). Other workers associated the effect of winter chilling requirement with a metabolic phase similar to vernalisation required in most cases for both stages of inflorescence induction and avocation (Hackett & Hartmann, 1967; Lavee, 1989, 1996; Troncoso et al., 2006). The range of low winter temperatures effective in fulfilling the chilling require-

ments, their amount, inductive period and daily cycle dynamics are not yet clear enough. In an older work, Hartmann & Whisler (1975) indicated that a daily gradual change between low and high temperatures is required for reproductive bud differentiation. They also showed that for some cultivars a moderate uniform temperature period of 12 °C was inductive for flower bud differentiation. Considerably more information is needed to evaluate the different daily and seasonally temperature cycles required for efficient although balanced, reproductive bud differentiation. Both the thermal conditions in the spring and early summer and the amount of developing young fruits on the tree are tightly linked in their effect on the potential level of bud differentiation for the yield in the following season (Lavee, 1989, 1996; Fernandez-Escobar et al., 1992; Cuevas et al., 1994; Fabri & Alerci, 1999; Baktir et al., 2004). But at the same time it is also obvious that it is not the only induction time, as in many cases buds which developed considerable later during the summer and autumn, might also be induced to differentiate into flower buds. Lately it has been shown by Lavee & Troncoso (unpubl.) that buds induced to grow in the autumn will develop vegetative shoots, while the same buds when forced later, in the winter, developed inflorescences.

As already indicated earlier, the vegetative growth following warm winters, causing reduced flower bud differentiation, will be abundant due to lack of developing fruit. Synchronized biannual bearing in such regions is normal and commonly expressed in olive industry. The temperatures in the spring, autumn and probably also during the summer are continuously involved in the induction and avocation processes governing flower bud differentiation. The thermal effect on the nature of bud development can be demonstrated in a simple flow diagram regardless of the endogenous metabolism involved (Fig. 2).

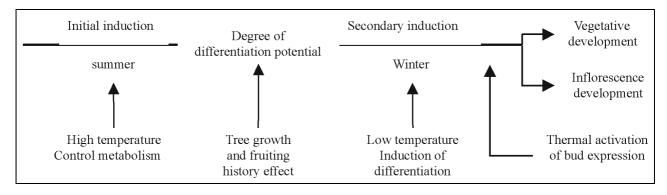


Fig. 2: A flow diagram of the effect of environmental conditions on the stages leading to reproductive and vegetative development in olive trees.

Fig. 2: Prikaz vpliva okoljskih dejavnikov na proces rodnosti in vegetativni razvoj oljčnih dreves.

# INTEGRATION OF ENDOGENOUS AND ENVIRONMENTAL EFFECTS LEADING TO ALTERNATE BEARING

Bud transformation leading to reproductive and vegetative stages is based on a continuous interaction between environmental inducing conditions and endogenous metabolic response. This integration is effect resulting in expression of different levels of growth and fruit production. The degree of phase expression determines the severances of alternate bearing and its long term development. As shown earlier, both endogenous and environmental factors are controlling the dynamics and level of each developmental phase of the tree leading to the degree of alternate bearing developing. The environmental conditions and particularly the temperature regime are instrumental in gene activation and repression initiating the metabolic activity leading to phase development and expression of the tree. Still, the developing organs were shown to have an independent controlling effect on the future phase development of the tree.

An attempt was made to summarize the timing and nature of involvement of both environmental and endogenous factors during the olive developmental cycle in consecutive 'on' and 'off' years (Fig. 3). The controlling factors, environmentally and or endogenously, on organ development are indicated. The circle is describing a biannual alternate bearing situation with the upper half representing the 'on' year and the lower half the 'off' one. Starting from the spring, the sequence of events during the 'on' year till the following spring has been recorded, from where the events in the 'off' year are described. Along the inner side of the circle, the timing of some of the major metabolic inductions was indicated.

Outside the circle, the sequences of the developmental stages are shown as well as their major dependence on environmental effects, endogenous induced conditions, or both.

It should be emphasized that each stage during the developmental cycle is affected by the previous one, but at the same time it also affects the potential expected expression of the one that follows. The level of fruit set will affect not only the differentiation ability for the following year but also the characteristics of the fruit in the present one such as fruit size, time of fruit maturation and, as a result, the rate of oil accumulation. A heavy yield is leading in the present year to small, late maturing fruit with a slower rate of oil accumulation. Under such conditions the negative affect of the developing fruit on bud differentiation for the following year is amplified, while the quality of the present fruit products is reduced. Alternate bearing has therefore a negative effect not only on labor distribution, oil mill capacity, storage requirement etc., but also on product quality in the 'on' year. Even on a two year quantitative basis the production of a fully alternating orchard is in the order of 10-20% lower than that of a non or only partially alternating one (Tab. 5). The commonly raised question of increasing alternate bearing and having half the orchard producing one year and the other half in the next one is usually problematic. In most regions, the annual climatic conditions are not uniform enough to allow such a stable long term production control. Once alternate bearing develops for the whole orchard, most of the expenses saved in cultivation and harvest costs (and even more) have to be spent on storage and increased oil mill capacity. But above all the probable quality reduction of at least part of the olive products is unfeasible for today's market.

Tab. 5: Production of fruit, oil yield and oil mill efficiency in alternate and uniformly bearing irrigated cv. Barnea olive orchards. (Calculations are based on 24 hr work and 1.5 t/hr machines.)

Tab. 5: Pridelek plodov, pridobitek olja in izkoristek oljarne za alternativno in redno rodne, namakane oljčne nasade cv. Barnea. (Izračuni so osnovani na 24-urnem obratovanju oljarne s kapaciteto predelave 1,5 t/uro.)

| Year              | Fruit yield (t/ha)        | Oil yield (t/ha) | Oil mill (hrs/100 ha) | Ha/machine |  |  |  |  |
|-------------------|---------------------------|------------------|-----------------------|------------|--|--|--|--|
|                   | Alternate bearing orchard |                  |                       |            |  |  |  |  |
| 'On'              | 22.0                      | 4.4              | 1467                  | 150        |  |  |  |  |
| 'Off'             | 3.0                       | 0.6              | 200                   | _          |  |  |  |  |
| Total for 2 years | 25.0                      | 5.0              | 1667                  | _          |  |  |  |  |
| •                 | Uniformly bearing orchard |                  |                       |            |  |  |  |  |
| 'On'              | 15.5                      | 3.1              | 1030                  | 225        |  |  |  |  |
| 'Off'             | 12.5                      | 2.5              | 836                   | _          |  |  |  |  |
| Total for 2 years | 28.0                      | 5.6              | 1866                  | _          |  |  |  |  |

# APPROACHES AND METHODS TO REDUCE ALTERNATE BEARING

Due to the high dependence of the processes leading to flower bud differentiation and fruit set on the environmental conditions, there are regions where alternate bearing can only be partially overcome. Drastic changes in climate common particularly in the eastern Mediterranean basin will re-induce biannual bearing even after or during continuous treatments for fruiting regulation. Without better understanding of the molecular control of bud differentiation, our ability to overcome alternate bearing in varying climates is usually temporary and incomplete. On the other hand, in well adopted stable climates alternate bearing can be reasonably well avoided or continuously minimized. Still, an unexpected incident might lead to synchronized alternate bearing also in usually favorable climatic conditions. Various methods have been developed and horticultural techniques modified to reduce or overcome alternate bearing.

Pruning is a one of the oldest and basic methods to control production in olive orchards. Pruning has a wide range of effects on the olive tree and olive orchards in general. It is instrumental in shaping tree form, controlling vegetative vigor, enhancing light penetration for regrowth and flower bud differentiation, adaptation to spray and harvest mechanization, etc. Although the olive is a sectorial tree with each scaffold performing rather individually, some overall effect on fruiting can be achieved by balancing the amount of fruiting wood in relation to the expected production. Opening the trees for effective light penetration into the canopy will increase the fruiting potential by enhancing flower bud differentiation. At the same time, the amount of available fruiting wood can be controlled. Applying a more severe pruning before the 'on' year will result in reducing the number of fruits by limiting the amount of fruiting wood. It will also cause initiation of new vegetative growth at the stamps of the pruned branches. This new vegetative growth could develop into fruiting wood for the following season if enough of the currently yielding wood had been removed. Towards the expected 'off' year pruning is than directed only to enhance light penetration, where the new developed canopy is too dense. In regions with a stable inductive climate, alternate bearing could be pretty much controlled by pruning. In regions with less stable climate and with slow growing cultivars not responding enough to pruning, additional methods, such as fruit thinning, will be needed.

Fruit thinning has an important impact on both fruit quality during the 'on' year itself and the fruiting potential for the following one. The amount of developing fruit on each tree and even scaffold is directly correlated with its size. Thus, excess production results in small fruit, which are of inferior value for table olives (Hart-

mann, 1952; Martin et al., 1980), and, as shown presently by Dag et al. (unpubl.), the amount of oil per fruit is reduced. This reduction is due to the smaller flesh/stone ratio in the small (within each cultivar) induced by excess fruiting (Lavee & Wodner, 2004). As by reducing fruit number their size and amount of mesocarp containing the oil is increased, even a significant removal of fruit in the 'on' year has only a moderate effect on reducing the final fruit and oil yields. The currently developing seeds were shown to have a negative effect on flower bud differentiation (Stutte & Martin, 1986b; Lavee, 1989) Reduction in fruit number on the trees reduces the number of seeds and minimizes their inhibiting effect on the fruiting in the following season. Fruit thinning is foremost a tool to improve yield quality during the 'on' year when it is performed (Hartmann, 1952). Severe thinning is at the same time a useful tool to reduce alternate bearing particularly in regions with unstable production. Thinning is performed by spray application of naphthaleneacetic acid (NAA) usually 10-20 days after full bloom once the degree of fruit-set has been established (Lavee & Spiegel, 1958, 1967; Martin et al., 1980). Late summer application of gibberellic acid (GA<sub>3</sub>) was shown to reduce flower bud differentiation and could be used to reduce the flowering towards the 'on' years (Fernandez-Escobar et al., 1992; Lavee & Haskal, 1993). The drawback of this method is its early application before the winter induction of flower bud differentiation and the climatic conditions for fruit-set during flowering. In northern Italy, a substituted vitamin based NAA ("66F") was reported to increase fruit-set when applied at the beginning of flowering (Bartolini et al., 1993). This might be an interesting approach for increasing fruit production in the 'off' year. Enhanced fruitset was also achieved by late winter application of gibberellin inhibitors of the triazol group, such as paclobutrazol, dichlorobutrazol etc., in addition to their effect on reduction or temporary reduction of vegetative growth (Prolingis & Voyiatzis, 1986; Lavee & Haskal, 1993; Rugini & Pannelli, 1993; Iannotta et al., 1999; Palliotti, 1999). The use of Pacloburazol or similar substances is presently studied both for growth and fruiting regulation in the new dense hedge-row developing orchards and in regular intensive orchards before the 'off' year. Harvest time should be considered in relation to alternate bearing. During the early stage of fruit maturation, harvest time has only a very slight effect on alternate bearing. However, late harvest at advanced or full fruit maturation has a significant negative affect on the conditions leading to the consecutive uniform crop development. Harvesting the fruit as early as maturation allows is of particular importance in the 'on' years to prevent amplifying the crop reduction in the 'off' ones. Avoidance of late harvest in the 'on' year is critical both for oil and table olives, shifting in 'on' years the product of the later toward early harvested fruit for green pickles.

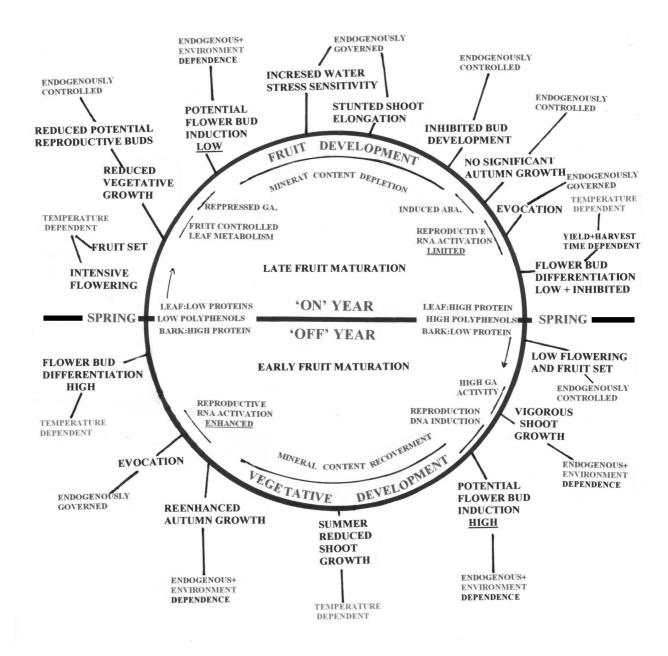


Fig. 3: A general scheme of the alternate bearing events, involvement of endogenous processes and interaction with the environment during an 'on' followed by an 'off' year (in black are the developmental stages, in red endogenous processes and in green environmental involvement).

Fig. 3: Splošna shema dogodkov alternativne rodnosti, vpliv notranjih dejavnikov in interakcija z okoljem v rodnem in nerodnem letu (s črno barvo so označenena razvojna obdobja, z rdečo notranji procesi in z zeleno vpliv okolja).

Girdling was found an efficient and feasible method for the reduction of alternate bearing in intensively cultivated table olive orchards. Girdling increased fruit set (Hartmann, 1950) and in some regions, mainly with warm winters, increased also the number of inflorescences when executed prior to the 'off' year (Lavee *et al.*, 1983). Girdling increased significantly the number of perfect flowers on the girdled scaffolds, widening the ra-

tio between perfect and male flowers (Levin & Lavee, 2005). To reduce alternate bearing, winter girdling is applied to half the scaffolds in one year and to the second half in the next. Thus, each scaffold of the tree is girdled every second year. This procedure has only a slight and some times no effect on the combined yield of the two years, but is efficient in reducing alternate bearing in the long run (Ben-Tal & Lavee, 1984). This

method is particularly useful for intensive irrigated orchards grown in regions with uniform weather conditions even when they were somewhat deficient in winter chilling. Continuous use of the alternate girdling method has no negative effect on the long term performance of the trees once applied to vigorous intensively grown ones. Girdling might cause scaffold decline when applied to weak and slow growing trees under extensive cultivation without irrigation in stress prone regions.

Finally, the possible involvement of nutrition, irrigation and fertigation in controlling alternate bearing has to be considered. Under normal balanced growing conditions, all aspects of intensification have very little influence on alternate bearing. Intensive olive cultivation increases production but does not significantly affect the alternate fruiting habit of the trees. Nutritional deficiencies and/or water stress might enhance alternate bearing. In such cases, nutritional or irrigation intervention would affect the level of biannual bearing as well. The use of these factors cannot be considered a significant mean to reduce alternate bearing. Spot-wise use of nutritional and water application are useful to avoid or correct alternate bearing in specific cases, when it was induced by an acute nutritional deficiency or water stress particularly during the early induction period.

#### **CONCLUSIONS**

Alternate bearing is a built-in character of olive trees. It is over all controlled by an interaction between vegetative growth and fruit load. The expression of alternate

bearing involves a wide range of changes in activation and repression of endogenous metabolic pathways. Environmental conditions are the main trigger to induce the metabolic changes leading to alternate bearing expression. A wide range of climatic events at different stages during the annual development of the olive tree might activate an array of metabolic pathways related to alternate bearing development. A continuous and complex interaction between the ambient temperatures, humidity and other environmental factors are involved in both the vegetative and reproductive development of olive buds. The information about the endogenous pathways and the genes involved in vegetative and reproductive bud differentiation and transformation is extremely limited. The nature of the signal transduction to initiate balanced or unbalanced vegetative/reproductive tree development is not yet known. Horticultural intervention via pruning, thinning, girdling and other cultural and nutritional means can reduce and even eliminate alternate bearing in regions with favorable and stable climatic conditions. Under more marginal and unstable environmental conditions, alternate bearing is most difficult to control and horticultural, often even drastic, means have to be reinitiated anew after each of the various extreme climatic events. For better understanding and controlling of alternate bearing, wide scale studies at the molecular level of the genes involved in olive flower bud initiation and development, and also the signals required for initiating the relevant metabolic pathways are urgently needed.

# IZMENIČNA RODNOST PRI OLJKAH (OLEA EUROPAEA)

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#### POVZETEK

Alternativna rodnost je zelo razširjen pojav pri številnih sadnih vrstah, ki povzroča veliko težav pri gojenju, trženju in ekonomičnosti proizvodnje. Pojav alternativne rodnosti je močno opazen tudi pri gojeni oljki (Olea europaea). Izražanje izmenične rodnosti pri oljkah vključuje številne spremembe v aktivaciji in zaviranju endogenih metabolnih poti. Stopnja izmenične rodnosti je močno odvisna od dejavnikov okolja in se lahko spreminja glede na klimatske okoliščine posameznega pridelovalnega območja. V delu so predstavljeni pomembnejši endogeni in okoljski dejavniki ter njihove interakcije, ki privedejo do alternativne rodnosti, in postopki, s katerimi lahko pojav zmanjšamo.

Ključne besede: Olea europaea, oljke, indukcija cvetnih brstov, izmenična rodnost, zmanjševanje izmenične rodnosti

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# EVALUATION OF THE ACIDITY IN SAMPLES OF VIRGIN OLIVE OIL FOR THE 1995-2005 PERIOD AND CORRELATION BETWEEN THE COLLECTED DATA AND THE PERFORMED SENSORY ANALYSIS

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# **ABSTRACT**

The acidity and organoleptic characteristics of virgin olive oil produced in Slovenian Istra were studied. The acidity of 4,871 samples of virgin olive oils was determined for the 1995-2005 period. 135 samples produced in crop year 2002/2003 with acidity below 0.2% (w/w) and 291 samples of crop year 2005/2006 were sensory evaluated. According to sensory evaluation in crop year 2002/2003, 128 out of 135 samples were classified as extra virgin olive oils, while 7 samples did not reach the specified requirements. In crop year 2005/2006, it was established that 71.5% of the analysed samples complied with extra virgin category, 24.4% with virgin and 4.1% with lampante. Time of harvest, storage conditions before processing and time from picking to processing all influenced the oil quality, whereas olive cultivars did not. Chemical and sensory analyses were performed according to the Commission Regulation (EEC) No 2568/91 and added annexes.

Key words: olive oil acidity, grading of oil, harvest, storage, processing, sensory analysis

# VALUTAZIONE DELL'ACIDITÀ DEI CAMPIONI DI OLIO D'OLIVA VERGINE NEL PERIODO 1995-2005 E CORRELAZIONE FRA DATI RACCOLTI E ANALISI SENSORIALE

# SINTESI

Gli autori hanno studiato l'acidità e le caratteristiche organolettiche dell'olio d'oliva vergine prodotto nell'Istria slovena. Hanno determinato l'acidità di 4871 campioni di olio d'oliva vergine del periodo 1995-2005. Centotrentacinque campioni prodotti nell'annata 2002/2003, con acidità al di sotto dello 0,2% (w/w) e 291 campioni dell'annata 2005/2006 sono stati valutati sensorialmente. Secondo la valutazione sensoriale 128 dei 135 campioni dell'annata 2002/2003, sono stati classificati come olii d'oliva extra-vergine, mentre 7 campioni non hanno soddisfatto tali criteri. Il 71,5% dei campioni dell'annata 2005/2006 sono risultati appartenenti alla categoria dell'olio d'oliva extra-vergine, il 24,4% a quella dell'olio d'oliva vergine e il 4,1% a quella dell'olio d'oliva lampante. Il periodo di raccolta, lo stoccaggio delle olive prima della lavorazione ed il tempo trascorso fra il raccolto e la lavorazione influenzano la qualità dell'olio, che non viene però intaccata dal tipo di cultivar. Le analisi chimiche e sensoriali sono state eseguite in conformità alla Regolazione della Commissione (EEC) No 2568/91 e degli allegati.

Parole chiave: acidità dell'olio d'oliva, classificazione degli oli, raccolto, stoccaggio, lavorazione, analisi sensoriale

# **INTRODUCTION**

Virgin olive oils are the oils obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration (Trade standard applying to olive oils and olive-pomace oils, 2003).

We show the results of acidity in crop years from 1995/1996 to 2005/2006. In this period, the free fatty acids were determined in 4,871 samples of oils from Slovenian Istra.

The quality evaluation of virgin olive oil is determined with chemical analyses and organoleptic assessment. Only the organoleptic assessment can determine the presence of the expected positive attributes of extra virgin olive oil and establish the eventual presence of defects.

We examined the results of the influence of the time of harvest, time from picking to processing, storage conditions before processing and production technology.

#### **MATERIAL AND METHODS**

We determined the free fatty acid amount and performed the sensory analysis in virgin olive oil samples produced in Slovenian Istra. The samples were collected in 11 oil mills operating in this area. In five oil mills, the oils are produced by the traditional non continuous production technology by presses and six oils mills adopted a modern continuous production technology. In this paper we consider the factors that influenced the acidity.

The data were acquired from the olive oil producers.

The acidity was determined in 4,871 olive oil samples collected from 1995 to 2005.

We performed the organoleptic assessment on 135 samples in crop year 2002/2003 with the acidity below 0.2% (w/w) and on 291 randomly chosen samples produced in crop year 2005/2006.

The acidity determination was performed using the SIST EN ISO 660 method. The acidity is a chemical parameter used to evaluate the quality of virgin olive oils and represents the free fatty acid amount expressed as percent content (w/w of oleic acid).

The sensory analysis was performed by the Slovene national panel for the organoleptic assessment of virgin olive oils according to the official method established in Commission regulation (EC) No 796/2002, Annex XII. The method can be used for grading virgin olive oils on the basis of fruitiness and intensity of defects.

Tasters must each smell and then taste the oil submitted for examination contained in the tasting glass, analysing their olfactory, gustatory, tactile and kinaesthetic perceptions and mark on the sheet the intensity of their perception of each negative and positive attribute.

#### **RESULTS**

The following paper shows the results of acidity of olive oil collected in the period from 1995 to 2005. In Table 1, we show the number on analysed samples of olive oil, the average acidity and the median acidity for the period from 1995 to 2005. In Table 2, the statistical data of acidity of olive oil for the period from 1995 to 2005 are presented.

Tab. 1: Number of analysed samples of olive oil, average and median acidity for the 1995–2005 period. Tab.1: Število analiziranih vzorcev, povprečna kislost in mediana kislosti v obdobju 1995–2005.

| Crop year | No. samples | Average acidity % (w/w) | Median acidity %<br>(w/w) |
|-----------|-------------|-------------------------|---------------------------|
| 1995/1996 | 382         | 0.81                    | 0.35                      |
| 1996/1997 | 303         | 0.62                    | 0.40                      |
| 1997/1998 | 113         | 1.02                    | 0.77                      |
| 1998/1999 | 267         | 0.27                    | 0.21                      |
| 1999/2000 | 366         | 0.44                    | 0.31                      |
| 2000/2001 | 389         | 0.59                    | 0.41                      |
| 2001/2002 | 477         | 0.29                    | 0.19                      |
| 2002/2003 | 596         | 0.61                    | 0.38                      |
| 2003/2004 | 412         | 0.18                    | 0.15                      |
| 2004/2005 | 664         | 0.28                    | 0.17                      |
| 2005/2006 | 902         | 0.18                    | 0.15                      |

Tab. 2: Statistical data of acidity of olive oil for the 1995–2005 period.

Tab. 2: Statistična obdelava podatkov za kislost v obdobju 1995–2005.

| Crop year | Min acidity % (w/w) | Max acidity % (w/w) | S    | CV (%) |
|-----------|---------------------|---------------------|------|--------|
| 1995/1996 | 0.06                | 7.83                | 1.11 | 136.26 |
| 1996/1997 | 0.07                | 10.37               | 0.82 | 133.76 |
| 1997/1998 | 0.14                | 4.77                | 0.82 | 80.38  |
| 1998/1999 | 0.05                | 2.40                | 0.24 | 90.17  |
| 1999/2000 | 0.09                | 2.94                | 0.41 | 91.90  |
| 2000/2001 | 0.08                | 8.20                | 0.70 | 117.27 |
| 2001/2002 | 0.07                | 3.49                | 0.29 | 100.58 |
| 2002/2003 | 0.07                | 7.20                | 0.78 | 127.00 |
| 2003/2004 | 0.07                | 2.71                | 0.17 | 91.67  |
| 2004/2005 | 0.06                | 9.01                | 0.52 | 184.45 |
| 2005/2006 | 0.05                | 1.47                | 0.13 | 76.28  |

In the past 10 years, the number of analyzed samples increased from 382 in 1995 to 902 in 2005. The results show that the lowest average acidity 0.18% (w/w) was determined in crop years 2003/2004 and 2005/2006, the minimal acidity 0.5% (w/w) in crop years 1998/1999 and 2005/2006, while the highest acidity 10.37% (w/w) was determined in crop year 1996/1997. Minimal and maximal determined acidities in the last crop year (2005/2006) were the lowest in the 10 past years.

# Comparison of crop years 1995/1996 and 2005/2006

In 1995, the acidity was determined in 382 samples of virgin olive oils, while in 2005, 902 samples were analysed. The number of samples increased proportionally to the production of olive oil. In 1995, 77% of samples complied with the request for extra virgin category, while in crop year 2005/2006, the number of samples

increased to 892 samples that represent 98.9% of the samples. As seen in Tables 1 and 2, the average and maximal acidity in crop year 2005/2006 comparing to crop year 1995/1996 decreased significantly.

The results of the influence of the time of harvest, time from picking to processing, storage conditions before processing and production technology on the acidity are presented in Tables 3 to 6.

The collected data show that the main olive oil production in crop year 1995/1996 was carried out in December (61% of samples), while in 2005, 71.3% of samples were produced in November. The average acidity was lower in crop year 2005/2006.

In 1995, most of the samples were processed in more than 48 hours. At that time, the olive producers used to pick and store the olive fruit for two or three weeks and subsequently delivered it to olive mills. Consequently the acidity of such samples was high.

Tab. 3: Influence of the time of harvest.

Tab. 3: Vpliv obdobja predelave.

| Crop year               | 1995 | 5/1996 | 2005/2006 |      |  |
|-------------------------|------|--------|-----------|------|--|
| Time of harvest         | NOV  | DEC    | NOV       | DEC  |  |
| % of samples            | 34.8 | 61.8   | 71.3      | 27.5 |  |
| Average acidity % (w/w) | 0.31 | 0.67   | 0.15      | 0.27 |  |
| Min. acidity % (w/w)    | 0.06 | 0.11   | 0.05      | 0.09 |  |
| Max. acidity % (w/w)    | 3.09 | 7.83   | 0.48      | 1.47 |  |

Tab. 4: Influence of the time from picking to processing.

Tab. 4: Vpliv časa skladiščenja pred predelavo.

| Crop year                       | 1995,    | /1996    | 2005/2006 |          |  |
|---------------------------------|----------|----------|-----------|----------|--|
| Time from picking to processing | ≤ 48 hrs | > 48 hrs | ≤ 48 hrs  | > 48 hrs |  |
| % of samples                    | 15.7     | 82.7     | 45.9      | 50.2     |  |
| Average acidity % (w/w)         | 0.14     | 0.95     | 0.14      | 0.17     |  |
| Min. acidity % (w/w)            | 0.06     | 0.08     | 0.05      | 0.06     |  |
| Max. acidity % (w/w)            | 0.77     | 7.83     | 1.03      | 1.47     |  |

Tab. 5: Influence of the storage conditions before processing.

Tab. 5: Vpliv načina shranjevanja.

| Crop year                            |                | 1995 | 5/1996             | 2005/2006     |       |               |                      |               |
|--------------------------------------|----------------|------|--------------------|---------------|-------|---------------|----------------------|---------------|
| Storage conditions before processing | Boxes Plateaus |      | Reticular<br>sacks | Other storage | Boxes | Pla-<br>teaus | Reticu-<br>lar sacks | Other storage |
| % of samples                         | 8.6            | 9.2  | 19.9               | 62.3          | 34.1  | 14.6          | 26.1                 | 25.1          |
| Average acidity % (w/w)              | 0.70           | 1.01 | 0.88               | 0.78          | 0.17  | 0.16          | 0.20                 | 0.19          |
| Min. acidity % (w/w)                 | 0.08           | 0.09 | 0.09               | 0.06          | 0.06  | 0.05          | 0.05                 | 0.06          |
| Max. acidity % (w/w)                 | 3.08           | 5.32 | 7.83               | 6.55          | 1.41  | 0.61          | 1.34                 | 1.47          |

Tab. 6: Influence of the production technology.

Tab. 6: Vpliv tehnologije predelave.

| Crop year               | 1995/             | /1996                      | 2005/2006         |                            |  |
|-------------------------|-------------------|----------------------------|-------------------|----------------------------|--|
| Production technology   | Continuous system | Non-cont. system (presses) | Continuous system | Non-cont. system (presses) |  |
| % of samples            | 47.9              | 46.8                       | 69.4              | 25.4                       |  |
| Average acidity % (w/w) | 0.67              | 0.83                       | 0.15              | 0.27                       |  |
| Min. acidity % (w/w)    | 0.06              | 0.10                       | 0.05              | 0.09                       |  |
| Max. acidity % (w/w)    | 7.83              | 7.32                       | 1.41              | 1.47                       |  |

Tab. 7: Grading of virgin olive oil.

Tab. 7: Razvrščanje deviškega oljčnega olja.

| Category               | Median of defects | Median of "fruity" |
|------------------------|-------------------|--------------------|
| Extra virgin olive oil | Me = 0            | Me > 0             |
| Virgin olive oil       | $0 < Me \le 2.5$  | Me > 0             |
| Lampante olive oil     | Me > 2.5*         | -                  |

<sup>\*</sup> or if the median of defects is less than or equal to 2.5 and the median of fruity is 0.

In 1995, only 8.6% and 9.2% of samples were stored respectively in boxes and plateaus. For the majority of samples (62.3%), we do not have any information about them. Nowadays more than 1/3 of samples are stored in boxes. In this case we also established that the average acidity was lower in crop year 2005/2006.

In 1995, the olive oil production was equally divided between continuous and non-continuous production technologies. Nowadays, only ¼ of the production is done on traditional systems with presses.

# Grading of virgin olive oils

The quality evaluation of virgin olive oil is determined with chemical analyses and organoleptic assessment. Only the organoleptic assessment can determine the presence of the expected positive attributes of extra virgin olive oil and establish the eventual presence of defects.

Samples of olive oils are graded as follows (Tab. 7) in line with the median of defects and the median for "fruity". By this the median of the negative attribute per-

ceived with the greatest intensity (Commission regulation (EC) No 796/2002, Annex XII) is understood.

# **Organoleptic assessment**

In 2002, the acidity was determined in 596 samples of virgin olive oils. The organoleptic assessment was performed in 135 samples with the acidity below 0.2% (w/w). In spite of lower acidity, seven samples did not comply with the request for extra virgin category. The determined defects were fusty, winy-vinegary and muddy sediment. Additionally, the results were statistically evaluated performing Duncan's test. There were no statistical significant differences between the data marked with the same index in the column in Tables 8 to 12.

The results of the influence of the time of harvest, time from picking to processing, storage conditions before processing, production technology and cultivars on the sensory attributes are presented in Tables 8 to 12.

Based on the statistical evaluation it results that the time of harvest did not influence the intensity of the

positive sensory attributes. It was observed that the sensory attributes of the oils produced in November were more intensive. Also, the negative attributes (the defects) did not depend on the time of harvest.

The results show that the time from picking to processing influenced the sensory attributes of virgin olive oils. The oils produced in 36 hours from picking were more bitter and pungent. There is a statistical significant difference between the samples.

The collected data show that 77 samples of olives were stored in boxes and plateaus. For 40 samples, we do not have any information about the storage condition before processing. In these samples, presence of the three observed defects (fusty, winy-vinegary and muddy

sediment) was determined at the same time. Statistically, the results are classified in the same group.

The results show that in crop year 2002/2003 the olive oil samples produced with a two-phase continuous system were more fruity, bitter and pungent if compared to the other production technology. The data were ranged in two groups (indexes <sup>a, b</sup>).

The statistical analysis shows that the production technology does not have any influence on the presence of defects fusty and winy-vinegary. The results are ranged in the same group. The negative attribute muddy sediment was determined in oils produced with the noncontinuous system by presses.

Tab. 8: Influence of time of harvest. Tab. 8: Vpliv obdobja predelave.

|                 |     |                             | Average intensity |                   |                                     |                   |                   |  |  |  |
|-----------------|-----|-----------------------------|-------------------|-------------------|-------------------------------------|-------------------|-------------------|--|--|--|
|                 |     | Positive attributes Defects |                   |                   |                                     |                   |                   |  |  |  |
| Time of harvest | No. | Fruity                      | Bitter            | Pungent           | Fusty Winy- Muddy vinegary sediment |                   |                   |  |  |  |
| NOV             | 134 | 3.75 <sup>a</sup>           | 3.18 a            | 4.07 <sup>a</sup> | 0.04 <sup>a</sup>                   | 0.10 <sup>a</sup> | 0.02 <sup>a</sup> |  |  |  |
| DEC             | 1   | 3.25 <sup>a</sup>           | 2.50 a            | 0.00 a            | 0.00 a                              | 0.00 a            |                   |  |  |  |

Tab. 9: Influence of the time from picking to processing. Tab. 9: Vpliv časa skladiščenja pred predelavo.

|                                 |     |                   | Average intensity           |                   |                                       |                   |                   |  |  |
|---------------------------------|-----|-------------------|-----------------------------|-------------------|---------------------------------------|-------------------|-------------------|--|--|
|                                 |     | Po                | Positive attributes Defects |                   |                                       |                   |                   |  |  |
| Time from picking to processing | No. | Fruity            | Bitter                      | Pungent           | t Fusty Winy- Mudd<br>vinegary sedime |                   |                   |  |  |
| ≤ 36 hrs                        | 100 | 3.84 <sup>a</sup> | 3.39 <sup>a</sup>           | 4.31 <sup>a</sup> | 0.05 a                                | 0.09 <sup>a</sup> | 0.03 <sup>a</sup> |  |  |
| > 36 hrs                        | 35  | 3.47 <sup>a</sup> | 2.55 <sup>b</sup>           | 3.34 <sup>b</sup> | 0.00°a                                | 0.11 a            | 0.00 a            |  |  |

Tab. 10: Influence of storage conditions before processing. Tab. 10: Vpliv načina shranjevanja.

|                    |     |                   | Average intensity |                   |         |                   |                   |  |  |
|--------------------|-----|-------------------|-------------------|-------------------|---------|-------------------|-------------------|--|--|
|                    |     | Po                | sitive attribu    | tes               | Defects |                   |                   |  |  |
| Storage condition  | No. | Fruity            | Bitter            | Pungent           | Fusty   | Winy-<br>vinegary | Muddy<br>sediment |  |  |
| Reticular sacks    | 16  | 3.73 <sup>a</sup> | 3.05 <sup>a</sup> | 3.98 <sup>a</sup> | 0.06 a  | 0.09 <sup>a</sup> | 0.00 a            |  |  |
| Non-defined        | 40  | 3.96 <sup>a</sup> | 3.45 a            | 3.99 <sup>a</sup> | 0.10 a  | 0.05 <sup>a</sup> | 0.08 a            |  |  |
| On the ground      | 2   | 3.75 <sup>a</sup> | 2.25 <sup>a</sup> | 3.13 <sup>a</sup> | 0.00 a  | 0.00 a            | 0.00 a            |  |  |
| Boxes and plateaus | 77  | 3.63 <sup>a</sup> | 3.08 <sup>a</sup> | 4.13 <sup>a</sup> | 0.00 a  | 0.12 a            | 0.00 <sup>a</sup> |  |  |

Tab. 11: Influence of the production technology.

Tab. 11: Vpliv tehnologije predelave.

|                       |     |                   | Average intensity |                   |                                     |         |        |  |  |  |
|-----------------------|-----|-------------------|-------------------|-------------------|-------------------------------------|---------|--------|--|--|--|
|                       |     | Po                | sitive attribu    | ites              |                                     | Defects |        |  |  |  |
| Production technology | No. | Fruity            | Bitter            | Pungent           | Fusty Winy- Muddy vinegary sediment |         |        |  |  |  |
| Presses               | 10  | 3.24 <sup>b</sup> | 2.28 <sup>b</sup> | 3.53 <sup>b</sup> | 0.00 a                              | 0.00 a  | 0.30 a |  |  |  |
| 2-phase               | 25  | 4.65 a            | 3.98 <sup>a</sup> | 5.01 <sup>a</sup> | 0.08 a                              | 0.14 a  | 0.00 b |  |  |  |
| 2.5-phase             | 100 | 3.57 b            | 3.06 <sup>b</sup> | 3.87 b            | 0.03 a                              | 0.10 a  | 0.00 b |  |  |  |

Tab. 12: Influence of the cultivars.

Tab. 12: Vpliv sorte.

|                                   |     |                             | Average intensity |                      |                   |                   |                   |  |  |  |
|-----------------------------------|-----|-----------------------------|-------------------|----------------------|-------------------|-------------------|-------------------|--|--|--|
|                                   |     | Positive attributes Defects |                   |                      |                   |                   |                   |  |  |  |
| Cultivar                          | No. | Fruity                      | Bitter            | Pungent              | Fusty             | Winy-<br>vinegary | Muddy<br>sediment |  |  |  |
| Ascolana                          | 1   | 4.00 <sup>a</sup>           | 1.00 <sup>a</sup> | 1.00 <sup>b</sup>    | 0.00 a            | 0.00 <sup>a</sup> | 0.00 a            |  |  |  |
| Istrska belica                    | 46  | 3.58 a                      | 3.36 a            | 4.26 a               | 0.07 <sup>a</sup> | 0.21 <sup>a</sup> | 0.07 <sup>a</sup> |  |  |  |
| Istrska belica 20%<br>Leccino 80% | 5   | 4.60 <sup>a</sup>           | 3.70 <sup>a</sup> | 4.30 <sup>a</sup>    | 0.00 a            | 0.00 a            | 0.00 a            |  |  |  |
| Istrska belica 40%<br>Leccino 60% | 1   | 3.00 <sup>a</sup>           | 2.50 <sup>a</sup> | 3.00 <sup>a. b</sup> | 0.00 a            | 0.00 a            | 0.00 a            |  |  |  |
| Istrska belica 50%<br>Leccino 50% | 29  | 3.86 <sup>a</sup>           | 3.28 <sup>a</sup> | 4.03 <sup>a</sup>    | 0.00 <sup>a</sup> | 0.00 <sup>a</sup> | 0.00 <sup>a</sup> |  |  |  |
| Istrska belica 70%<br>Leccino 30% | 4   | 3.94 <sup>a</sup>           | 3.06 <sup>a</sup> | 3.69 a. b            | 0.00 a            | 0.00 a            | 0.00 <sup>a</sup> |  |  |  |
| Istrska belica 90%<br>Leccino 10% | 2   | 2.75 <sup>a</sup>           | 2.75 <sup>a</sup> | 3.75 <sup>a. b</sup> | 0.00 a            | 0.00 a            | 0.00 a            |  |  |  |
| Leccino                           | 8   | 4.09 <sup>a</sup>           | 2.28 <sup>a</sup> | 3.31 <sup>a. b</sup> | 0.00 a            | 0.00 <sup>a</sup> | 0.00 a            |  |  |  |
| Mix of cultivars                  | 38  | 3.72 a                      | 3.15 <sup>a</sup> | 4.18 a               | 0.05 <sup>a</sup> | 0.09 <sup>a</sup> | 0.00 <sup>a</sup> |  |  |  |
| Oblica                            | 1   | 3.00 <sup>a</sup>           | 1.00 <sup>a</sup> | 1.50 <sup>a. b</sup> | 0.00 a            | 0.00 <sup>a</sup> | 0.00 a            |  |  |  |

Tab. 13: Number of virgin and lampante olive oil samples in crop year 2005/2006.

Tab. 13: Razvrščanje vzorcev letnika 2005/2006 v kategorijo deviško olje in oljčno olje lampante.

| Category           | Median of defects | Total<br>samples | Fusty | Musty | Muddy<br>sediment | Winy-<br>vinegary | Rancid |  |  |  |
|--------------------|-------------------|------------------|-------|-------|-------------------|-------------------|--------|--|--|--|
|                    | uelects           | samples          |       |       | No. sample        | s                 |        |  |  |  |
| Virgin olive oil   | 0 < Me ≤ 2.5      | 71               | 33    | 1     | 10                | 16                | 9      |  |  |  |
| Lampante olive oil | Me > 2.5          | 12               | 3     | 2     | 4                 | 2                 | 5      |  |  |  |

Based on the statistical analysis of the results in crop year 2002/2003, the sensory attributes fruity and bitter do not depend on the olive cultivars. The most pungent samples were those produced from the mix of cultivars lstrska belica and Leccino in proportion 20%: 80%, followed by the sample produced from the cultivar lstrska belica. The two cultivars, Istrska belica and Leccino, differ in the amount of biophenols that influenced

the bitter and pungent sensation. The biophenols amount is higher in Istrska belica cultivar. Based on this consideration, we expected the sample Istrska belica to be more pungent.

In 2005, the organoleptic assessment was performed on 291 samples. 208 of them were without defects and complied with the category extra virgin olive oil, 71 samples were ranged in the virgin category, while 12

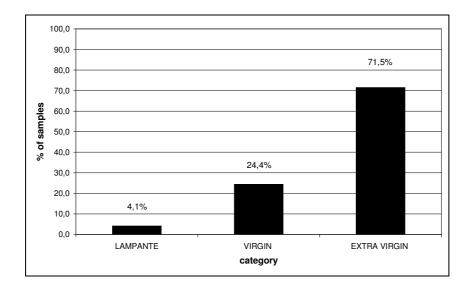


Fig. 1: Grading of samples in categories (% of samples) collected and analysed in crop year 2005/2006. Sl. 1: Razvrščanje vzorcev letnika 2005/2006 v kategorije (% vzorcev).

samples were classified as lampante olive oil. Figure 1 shows the proportion of samples that was assigned to each category.

# Extra virgin olive oil

The fruity intensity of extra virgin olive oil samples was medium, in the range from 2.5 to 6.5. The fruity of 49.5% of samples was 4–4.5. The bitter and the pungent sensations were of medium intensity (4–5) as well, in 47.8% and 34.1% of samples respectively. The bitter was in the range from 2 to 7, the pungent from 3 to 6.5.

# Virgin olive oil

The negative attribute fusty was predominant in 33 samples, muddy sediment in 16 samples, 10 samples were winy-vinegary and 9 samples were rancid. The attribute musty was determined in one sample, in which the predominant defect was fusty. The results are given in Table 13.

# Lampante olive oil

The median of defects was above 2.5. In 4 samples the defect "muddy sediment" was noted, "fusty" in 3 samples, "musty" in 2 samples, whereas in 2 samples the predominant defect was winy-vinegary. The assessors, however, also determined muddy sediment and fusty of low intensity. The defect rancid was also determined, but was not predominant. The results are given in Table 13.

#### **DISCUSSION**

It was established that in 1995 77% of samples complied with the request for extra virgin category. According to the legislation for extra virgin olive oil at that time (Commission Regulation (EEC) No 2568/91), max. 1% (w/w) of acidity was allowed. The average acidity of the samples was 0.81% (w/w). The minimal determined acidity was 0.06% (w/w), the maximum was 7.83% (w/w). In ten years, the average acidity has decreased and consequently the number of samples that comply with the criteria for extra virgin category has increased.

In the last crop year 2005/2006, we determined free fatty acids in 902 samples. The average acidity was 0.18% (w/w). The minimal determined acidity was 0.05% (w/w), the maximum was 1.47% (w/w).

892 samples that represent 98.9% of samples complied with the criteria for extra virgin category. According to the current legislation (Commission Regulation (EC) No 1989/2003), the maximum allowed acidity for this category is 0.8% (w/w).

The quality evaluation of virgin olive oil is determined with chemical analyses and organoleptic assessment. Only the organoleptic assessment can determine the presence of the expected positive attributes of extra virgin olive oil and establish the eventual presence of defects. Hypothetically, samples of oils with lower acidity are without any defects. Such samples stored in proper conditions can maintain an acceptable quality even after a year.

The second part of the research was the sensory analysis of virgin olive oil samples. We performed the organoleptic assessment on 135 samples, which repre-

sent 22.5% of the analysed samples in crop year 2002/2003 with the acidity below 0.2% (w/w). In spite of the low acidity, seven samples had defects of fusty, winy-vinegary and muddy sediment. The perceived intensity of defects was low; therefore the samples were ranged in virgin olive oil category. The positive attributes fruity, bitter and pungent of extra virgin olive oils were medium intensive. The results of the sensory analysis of olive oils crop year 2002/2003 show that there are no significant differences between the samples. In spite of that, the influence of some factors was determined.

Olive oils produced in November were more bitter and pungent even if there were no statistical significant differences in the single sample produced in December.

The sensory analysis indicated that the time from picking to processing influenced the perceived intensity of the sensory attributes of the oils. Oils produced in 36 hours were more bitter and pungent, and differed form the oils produced in more than 36 hours.

The intensity of sensory attributes of the oils was not influenced by olive cultivars. We established that there were no significantly differences in oils produced from olives stored in suitable conditions before processing.

In crop year 2002/2003, the sensory analysis indicated that oils produced by the two-phase continuous system were more fruity, bitter and pungent compared to other production technologies. Defects fusty and winy-vinegary were found in samples produced by the continuous system, while the defect muddy sediment was determined in the only olive oil sample produced by presses (non-continuous system).

The positive and eventually negative sensory attributes are influenced due to the prolonged contact of oil

and water by the malaxation of olive pasta (Lercker, 2003).

We performed a similar research on some samples of the crop year 2005/2006. 71.5% of the analysed samples complied with extra virgin category, 24.4% with virgin and 4.1% with lampante.

#### **CONCLUSIONS**

In 1995, 77% of samples complied with the request for extra virgin category, while in crop year 2005/2006 the number of samples increased to 892 samples that represent 98.9% of the samples.

Hypothetically, samples of oils with lower acidity are without any defects. Such samples stored in proper conditions can maintain an acceptable quality even after a year. Only the organoleptic assessment can determine the presence of expected positive attributes of extra virgin olive oil and establish the eventual presence of defects. We performed organoleptic assessment on 135 samples, which represent 22.5% of the analysed samples in crop year 2002/2003 with the acidity below 0.2% (w/w). In spite of the low acidity, seven samples had defects of fusty, winy-vinegary and muddy sediment. In crop year 2005/2006, we established that 71% of the analysed samples complied with extra virgin category, 24.4% with virgin and 4.1% with lampante. Time of harvest, time from picking to processing and storage conditions before processing all influenced the acidity and the sensory attributes of virgin olive oils. The intensity of sensory attributes of the oils was not influenced by olive cultivars.

# PREGLED ANALIZE KISLOSTI VZORCEV DEVIŠKEGA OLJČNEGA OLJA V OBDOBJU 1995-2005 IN KORELACIJA TEH PODATKOV Z OPRAVLJENIMI SENZORIČNIMI ANALIZAMI

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#### **POVZETEK**

Raziskava obsega preučevanje kislosti in senzoričnih značilnosti deviškega oljčnega olja Slovenske Istre. Določili smo kislost 4871 vzorcev v obdobju 1995-2005. Senzorično je bilo ocenjenih 135 vzorcev letnika 2002/2003 s kislostjo pod 0,2 ut. % in 291 vzorcev letnika 2005/2006. 128 vzorcev letnika 2002/2003 je bilo ekstra deviške kakovosti, le pri sedmih vzorcih so bile ugotovljene napake. 71,5% vzorcev letnika 2005/2006 je bilo brez senzoričnih napak in razvrstili smo jih v kategorijo ekstra deviško oljčno olje, 24,4% v deviško oljčno olje in 4,1% v lampante oljčno olje. Ugotovili smo, da obdobje predelave, čas skladiščenja pred predelavo in način shranjevanja oljk vplivajo na kakovost olja, same sorte pa ne vplivajo. Kemijske analize in senzorično ocenjevanje smo opravili v skladu z Uredbo komisije (EGS) št. 2568/91 in dopolnilnimi aneksi.

Ključne besede: kislost oljčnega olja, razvrščanje olja, obiranje, shranjevanje, predelava, senzorična analiza

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# EVALUATION OF MEASUREMENT UNCERTAINTY FOR THE METHODS OF ANALYSIS USED TO ASSESS THE CHARACTERISTICS OF OLIVE OIL AND OLIVE-POMACE OIL FROM COMMISSION REGULATIONS EEC 2568/91 AND EC 1989/2003 – A CASE SAMPLE EVALUATING THE MEASUREMENT UNCERTAINTY FOR PEROXIDE VALUE

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# **ABSTRACT**

Metrology has been present in our scientific knowledge for a long time, but mostly in physical measurements. Chemical measurement can be defined as a comparison of a quantity of measurand and relating it to a unit (e.g. mol/kg). When expressing a result of a measurement, the problem of traceability, validation and the measurement uncertainty (MU) evaluation must be challenged. It is well known that MU is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. The EEC document 2568/91 with its annexes gives the methods of analysis to assess the characteristics of olive oils and olive-pomace oils with given limits. The EC document 1989/2003 gives the scheme, the algorithm, the pathway, the decision tree to differentiate between various types of olive oils using the particular determinations based on limits. The major lack of these EC methods is the non-existence of validation parameters, which are crucial in determining the MU (and in accreditation of a laboratory). There are several ways of evaluating (better term than calculating) the MU: with model equation, with use of a certified reference material (CRM) and with participation in a proficiency testing (PT) scheme. MU for a method of analysis is inherent to a laboratory and can serve as a tool for "measuring" the quality of a laboratory. Unfortunately, there are practically no CRM's in the olive oil testing field. The only way to achieve a usable MU is through participation in PT schemes.

Key words: CRM, EEC 2568/91, EC 1989/2003, metrology, olive oil, PT scheme, uncertainty

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# VALUTAZIONE DELL'INCERTEZZA DI MISURA DI METODI DI ANALISI USATI PER ACCERTARE LE CARATTERISTICHE DEGLI OLI D'OLIVA E DEGLI OLI DI SANSA D'OLIVA SECONDO I REGOLAMENTI CEE 2568/91 ED CE 1989/2003 – ESEMPIO DI VALUTAZIONE DELL'INCERTEZZA DI MISURA PER DELL'INDICE DI PEROSSIDO

#### SINTESI

La metrologia è parte della nostra conoscenza scientifica ormai da lungo tempo, specialmente nelle misurazioni fisiche. Le misurazioni chimiche possono venir definite come confronto fra la quantità della sostanza misurata e la relativa unità di misura (per es. mol/kg). Quando viene espresso il risultato di una misurazione, i problemi di rintracciabilità, convalida e incertezza di misurazione (MU) devono venir posti in primo piano. È ben noto che MU sia un parametro associato al risultato della misurazione che caratterizza la dispersione dei valori, che possono venir ragionevolmente attribuiti alla sostanza misurata. Il documento CEE 2568/91, con i suoi allegati, fornisce i metodi di analisi usati per valutare le caratteristiche degli olii d'oliva e degli olii d'oliva residui in confronto ai limiti prestabiliti. Il documento CE 1989/2003 fornisce lo schema, l'algoritmo, il percorso da seguire nel processo di differenziazione dei vari tipi di olii d'oliva, usando particolari determinazioni basate sui limiti. La maggior lacuna di tali metodi EC consiste nella non-esistenza di parametri convalidati, cruciali nella determinazione di MU (e per l'accreditamento del laboratorio). Ci sono vari modi di valutare (ossia calcolare) MU: con un'equazione modello, con l'uso di materiali di riferimento certificati (CRM) e con la partecipazione allo schema di verifica empirica competente (PT). MU come metodo di analisi è inerente al laboratorio e può venir utilizzato come strumento per la "misurazione" della qualità del laboratorio. Purtroppo, praticamente non ci sono CRM nel campo di verifica empirica competente.

Parole chiave: CRM, CEE 2568/91, CE 1989/2003, metrologia, olio d'oliva, schema PT, incertezza

# INTRODUCTION

"Nosotros (la indivisa divinidad que opera en nosotros) hemos soñado el mundo. Lo hemos soñado resistente, misterioso, visible, ubicuo en el espacio y firme en el tiempo; pero hemos consentido en su arquitectura, tenues y eternos intersticios de sinrazón para saber que es falso." We believe this quote by Argentinean writer, poet and critic J. L. Borges (Borges, 1932), can serve as a good starting point for the introduction of the topic of this article, dealing with the measurement uncertainties in the field of olive oil characteristics assessment.

The term measurement uncertainty must be introduced from the metrological point of view and that is why some basic terms and definitions will be presented. Metrology is simply the Science of measurement, measurement being the "set of operations having the object of determining a value of a quantity" (MIRS, 2001), and it is nowadays part of every scientific process in all spheres of science, including chemistry and its role in the sustainable development of our Earth (Meinrath & Kalin,

2005). Consequently, chemical measurement is a comparison, quantification of the measurand - "particular quantity subject to measurement" (EURACHEM, 1999) and its traceability to the unit. There are several important differences between physical and chemical measurement and they arise from the fact "that in physical measurement the issue is to compare quantities (e.g. lengths of different tables) traceable to a unit (e.g. metre) and in chemical measurement the issue is to compare an amount of analyte (e.g. content of DDT in meat) traceable to a unit (e.g. mol/kg)", thus giving the importance to the calibration of the instruments in the physical measurements and to the measurement procedure calibration in chemical measurements (Taylor et al., 2003). A chemical measurement has many crucial steps, among them sample preparation and sampling, preparation and physical calibration of the instrument, measurement, data handling and reporting of the results. Another term that must be understood is traceability, which "is a property of the result of a measurement or the value of a standard whereby it can be related to stated references,

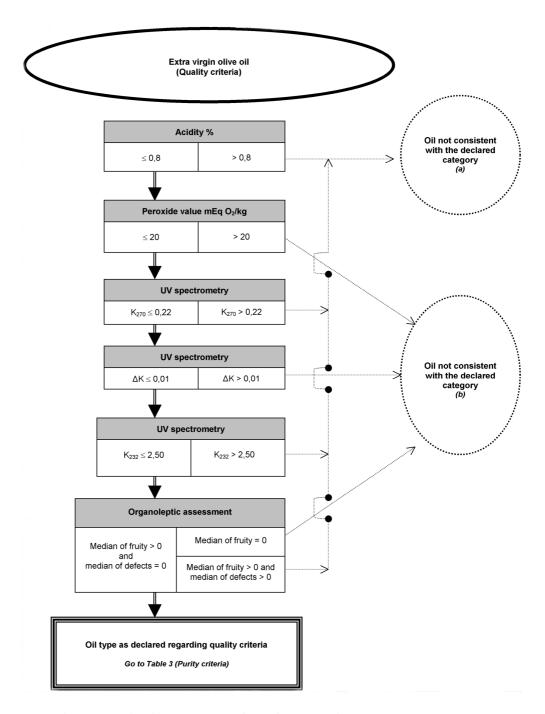


Fig. 1: Quality criteria for the extra virgin olive oil (EC 1989/2003, 2003). Sl. 1: Kakovostni parametri za ekstra deviško oljčno olje (EC 1989/2003, 2003).

usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties." (ISO VIM, 1993). The next step in the proper understanding of the results of the chemical measurement is validation (of the method, of the instrument, of the software tools used ...), defined as "the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use

are fulfilled" (ISO/IEC 17025, 1999). Every (chemical) measurement result must be accompanied by "parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand" – measurement uncertainty (ISO VIM, 1993).

Measuring uncertainty, which must accompany the result of a chemical measurement, is always connected

to the measurand, to the matrix characteristics, to the measuring – analytical method and concentration range as well. We deliberately did not want to enter into measuring uncertainty details like coverage factor, expanded uncertainty etc., but have instead tried to make the metrological concept of the measuring uncertainty as clear as possible.

The document "Regulation (EEC) No. 2568/91" (EEC 2568/91, 1991) and its amends give *inter alia* the methods of analysis and the limit values needed to assess the characteristics of olive and olive-pomace oil. The methods describe the way of performing chemical measurements in the quality field (*i.e.* the results show the quality of the oil in a declared category – *e.g.* extra virgin olive oil) and these are: acidity, peroxide value, UV spectrometry ( $K_{270}$ ,  $\Delta K$ ,  $K_{232}$ ) and in the purity field (the results show the consistency within declared category): 3,5-stigmastadienes, trans fatty acids (oleic, linoleic and linolenic), fatty acids content,  $\Delta ECN42$ , sterols composition and total sterols, erythrodiol + uvaol, waxes, saturated fatty acids in the 2-position, alcohols.

The document "Commission Regulation (EC) No. 1989/2003" (EC 1989/2003, 2003) gives the algorithm, the decision tree helping the assessor (analyst, laboratory manager, official inspector) in finding or judging the olive oil sample consistency with the declared category. There is another crucial value influencing the overall quality assessment – sensorial (organoleptic) assessment, but not being a chemical measurement it will not be discussed herewith. Figures 1 and 2 show the decision tree for the quality and purity criteria for extra virgin olive oil.

These methods of analysis have a big disadvantage in the light of the metrological point of view – they lack the validation parameters, which are the crucial point for many legal aspects of the testing laboratory comprising the accreditation documents. And here a sort of *circulus vitiosus* arises: how can a laboratory possibly come to these validation data? The only, although tedious way is to make the so called in-house methods and link them through traceable determinations and control samples to the mentioned methods of analysis from the document. And secondly, how to reach/determine all the needed (metrological and accreditation demand) uncertainty values for all methods of analysis?

It is well known that several approaches exist, some come from the uncertainty budget construction based on the model equations (Dehouck *et al.*, 2003; Tartacca *et al.*, 2003 (the only one dealing exclusively with olive

oils spectrophotometric determination); Clare, 2005; Populaire & Gimenez, 2006; Sooväli et al., 2006), some use Certified Reference materials (CRM) (Gluschke et al., 2005), some use data from the Inter Laboratory Comparisons (ILS) and/or Proficiency Testing (PT) schemes. A CRM is defined as "Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence" (ISO VIM, 1993). Several definitions exist (Majcen, 2003) and lately there is a need for somehow more accurate definitions of CRM's (Emons et al., 2006). In the olive oil field there are, unfortunately, practically no CRM's. There are ways, which a lab can take to solve this problem with the aid of control samples (Venelinov & Sahuquillo, 2006), but we cannot use this knowledge and experience at the beginning of the uncertainties evaluation - when the lab desperately needs it. All the approaches of uncertainty evaluation are to a minor or greater extent connected to the validation data (Hierro, 2003 (validation and uncertainty parameters for olive oils methods); Diaz et al., 2004). It seems the only valid approach is the use of ILS/PT schemes. In the light of accreditation the PT schemes ("A periodic assessment of the performance of individual laboratories and groups of laboratories that is achieved by the distribution by an independent testing body of typical materials for unsupervised analysis by the participants." (EURACHEM, 1999) have somewhat "stronger" impact on the measurement uncertainty evaluation compared to the ILS ones. The "uncertainty" within the data used for the uncertainty evaluation arises from the possible inaccurate determination of the reference value, which is in the most cases assigned value (Wong, 2005; Patriarca et al., 2006; Švegl et al., 2006; Thompson & Wood, 2006; Thompson et al., 2006), from the number and fitness of participants and from the quality of the organiser of the PT scheme. In the olive oil area there are, as far as we know, only 2 relevant organisers. One is the "International Olive Council" covering all the mentioned methods of analysis. It runs the PT scheme once a year and involves from 50-60 participants, which must be nationally approved labs within their respective countries. The other organiser is French "Bipea", which does not cover all the determinations and has somehow smaller number of participating labs and covers all types of fats on a monthly basis (10 samples per year).

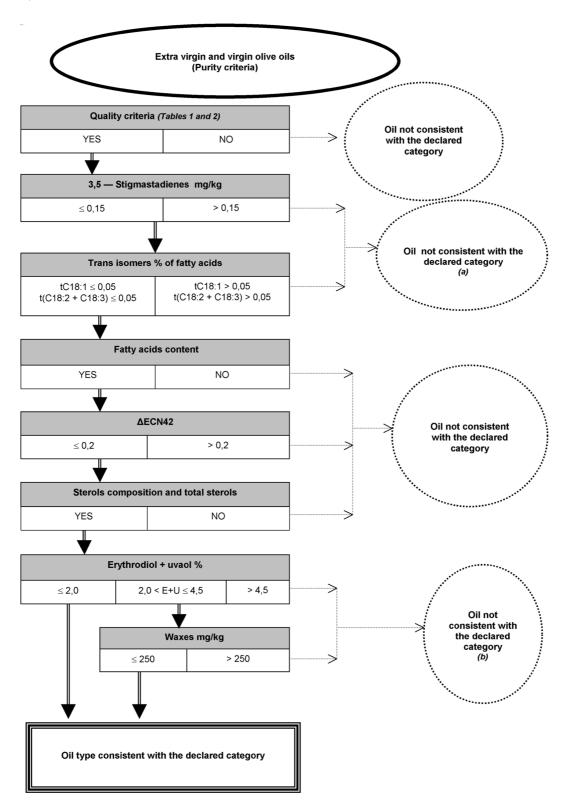


Fig. 2: Purity criteria for the extra virgin olive oil (EC 1989/2003, 2003). Sl. 2: Parametri pristnosti za ekstra deviško oljčno olje (EC 1989/2003, 2003).

#### **MATERIAL AND METHODS**

For the purpose of this paper, only one method of analysis was chosen – determination of peroxide value - and the PT schemes' data for this method's results were used to evaluate the MU.

**PT Schemes.** The PT schemes our laboratory participated in were two:

- PT scheme organised by the International Olive Council (IOC), Príncipe de Vergara 154, 28002 Madrid (Spain);
- PT scheme organised by BIPEA, 6-14 avenue Louis Roche, F-92230 Gennevilliers, FRANCE; COFRAC accredited.

**Samples.** The determination of Peroxide value was performed in 9 oil samples in the years 2003, 2004 and 2005. The samples were as follows:

- IOC: sample 23-2003 (year 2003), 24-2004 (year 2004), 25-2004 (year 2004), 28-2005 & 29-2005 (year 2005);
- Bipea: sample B 21 358 (year 2004), B 21 359 (year 2004), B 21 360 (year 2004) & B 21 364 (year 2005).

*Methods.* The methods used were as follows:

- Method of Analysis Determination of the peroxide value, EEC No. 2568/91, Annex III (EEC 2568/91, 1991);
- Statistical tools:
  - ISO 5725-2 (1994) Accuracy (trueness and precision) of measurement methods and results—Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method. International Organization for Standardization (ISO), Geneva, Switzerland (ISO 5725-2, 1994);
  - ISO/DIS 13528 (2002) Statistical methods for use in proficiency testing by interlaboratory comparisons. International Organization for Standardization (ISO) Geneva, Switzerland (ISO/DIS 13528, 2002).
- Guides:
  - EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement (EURACHEM, 2000);
  - Handbook for calculation of measurement uncertainty in environmental laboratories (Nordtest, 2004).

The method of analysis – determination of peroxide value is a redox titration where peroxides (hydro peroxides) present in the oil that is dissolved in acetic acid and chloroform react with potassium iodide oxidising it to free iodine. The iodine is titrated with standardized sodium thiosulfate solution and the peroxide value expressed in meq (mmol) of active oxygen/kg oil.

The method of analysis was used to measure – determine the Peroxide value in the samples. Guides (Eurachem, 2000; Nordtest, 2004) were used to construct the uncertainty budget for the Peroxide value determination. The statistical tools - 2 ISO standards (ISO 5725-

2, 1994; ISO/DIS 13528, 2002) helped us in calculating the robust means and robust standard deviations for each PT scheme and in calculating the intra-laboratory standard deviation.

## **RESULTS AND DISCUSSION**

The measurement uncertainty evaluation of the measurand A is a property of the laboratory lab performing the chemical measurement based on the method M and is generally based on two relatively independent sources – the method M, under which the chemical measurement is made, and the laboratory lab that performs the chemical measurement. The combined uncertainty of the chemical measurement of measurand A -  $u_A$  is function of the uncertainty  $u_{lab}$  arising from the method and uncertainty  $u_{lab}$  arising from the lab.  $u_A$  is calculated from the equation:

$$u_A = \sqrt{(u_M)^2 + (u_{lab})^2}$$
.

There are several ways of calculating u<sub>M</sub>:

- a) from the CRM
- b) from the model equation after constructing the uncertainty budget
- c) from method validation data
- d) from the method validation data combined with PT schemes' results – from the organisers side (Hierro, 2003)
- e) from the single or few PT schemes' results from the participants side and  $u_{lab}$ :
- f) from the CRM
- g) from the PT scheme
- h) from the recovery data

Two different measurement uncertainty evaluations (together with respective calculations and results) were used in our lab, and we show them briefly herewith.

# First type of evaluation

At the beginning of our lab's activity we had the lack of validation data, we were not in possession of any CRM's and we participated in only one PT scheme (1 single determination). We were forced to use the combination of b) and g) calculation approach. We used the model equation

$$PV = 3000 \times \frac{(V - V_{b1}) \times V_p \times m_{_0} \times P}{m \times M_{\text{KIO}_3} \times V_{0} \times V_b}$$

for the calculation of the peroxide value. Each variable's standard uncertainty was calculated according to EU-RACHEM guide's (EURACHEM, 2000) using the equation for the standard uncertainty of the sum. The value (V-V<sub>bl</sub>) was expressed as DV and its uncertainty was calculated likewise. The repeatability element (REP) was calculated from the validation data (Tab. 1).

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With the aid of the EURACHEM guide's (EURACHEM, 2000), basic equation

$$u_{M}(y) = y\sqrt{\left(\frac{u(DV)}{DV}\right)^{2} + \left(\frac{u(V_{p})}{V_{p}}\right)^{2} + \left(\frac{u(m_{0})}{m_{0}}\right)^{2} + \left(\frac{u(P)}{P}\right)^{2} + \left(\frac{u(m)}{m}\right)^{2} + \left(\frac{u(M_{KIO_{3}})}{M_{KIO_{3}}}\right)^{2} + \left(\frac{u(V_{0})}{V_{0}}\right)^{2} + \left(\frac{u(V_{p})}{V_{p}}\right)^{2} + \left(\frac{u(REP)}{REP}\right)^{2} \dots }$$

and spreadsheet calculation modification by Kragten (Ellison, 2005) for the calculation of the differentiation:

$$u_{M}(y, x_{i}) \approx y(x_{1}, x_{2}, ...(x_{i} + u(x_{i}))...x_{n}) - y(x_{1}, x_{2}, ...x_{n})$$

the result for combined standard uncertainty was evaluated as  $u_A = u_{PV} = 0.0617$  mmol/kg.

Tab. 1: The spreadsheet results for the calculation of the combined standard uncertainty for PV. Tab. 1: Rezultati pregledničnega izračuna sestavljene standardne negotovosti za PŠ.

|                 |             | DV (mL)      | Vp (mL)  | mo (mg)  | P (mol/mol) | m (g)    | M (g/mol) | V0 (mL)  | Vb       | REP      |
|-----------------|-------------|--------------|----------|----------|-------------|----------|-----------|----------|----------|----------|
|                 | Value       | 9,9756       | 10,0046  | 50,0000  | 1,0004      | 5,0000   | 214,0010  | 5,6000   | 250,0082 | 1,0000   |
|                 | Uncertainty | 0,0130       | 0,0102   | 0,0099   | 0,0011      | 0,0000   | 0,0005    | 0,0088   | 0,1517   | 0,0056   |
|                 |             |              | _        |          |             |          |           |          |          |          |
| DV (mL)         | 9,9756      | 9,9886       | 9,9756   | 9,9756   | 9,9756      | 9,9756   | 9,9756    | 9,9756   | 9,9756   | 9,9756   |
| Vp (mL)         | 10,0046     | 10,0046      | 10,0148  | 10,0046  | 10,0046     | 10,0046  | 10,0046   | 10,0046  | 10,0046  | 10,0046  |
| mo (mg)         | 50,0000     | 50,0000      | 50,0000  | 50,0099  | 50,0000     | 50,0000  | 50,0000   | 50,0000  | 50,0000  | 50,0000  |
| P (mol/mol)     | 1,0004      | 1,0004       | 1,0004   | 1,0004   | 1,0015      | 1,0004   | 1,0004    | 1,0004   | 1,0004   | 1,0004   |
| m (g)           | 5,0000      | 5,0000       | 5,0000   | 5,0000   | 5,0000      | 5,0000   | 5,0000    | 5,0000   | 5,0000   | 5,0000   |
| M (g/mol)       | 214,0010    | 214,0010     | 214,0010 | 214,0010 | 214,0010    | 214,0010 | 214,0015  | 214,0010 | 214,0010 | 214,0010 |
| V0 (mL)         | 5,6000      | 5,6000       | 5,6000   | 5,6000   | 5,6000      | 5,6000   | 5,6000    | 5,6088   | 5,6000   | 5,6000   |
| Vb              | 250,0082    | 250,0082     | 250,0082 | 250,0082 | 250,0082    | 250,0082 | 250,0082  | 250,0082 | 250,1599 | 250,0082 |
| REP             | 1,0000      | 1,0000       | 1,0000   | 1,0000   | 1,0000      | 1,0000   | 1,0000    | 1,0000   | 1,0000   | 1,0056   |
|                 |             |              |          |          |             |          |           |          | •        |          |
| PV              | 9,9971      | 10,0102      | 10,0073  | 9,9991   | 10,0081     | 9,9971   | 9,9971    | 9,9814   | 9,9911   | 10,0530  |
| u(y,xi)         |             | 0,0131       | 0,0102   | 0,0020   | 0,0110      | 0,0000   | 0,0000    | -0,0158  | -0,0061  | 0,0559   |
| u(y)2, u(y,xi)2 | 0,0038      | 0,0002       | 0,0001   | 0,0000   | 0,0001      | 0,0000   | 0,0000    | 0,0002   | 0,0000   | 0,0031   |
| index           |             | 0,0449       | 0,0271   | 0,0010   | 0,0317      | 0,0000   | 0,0000    | 0,0652   | 0,0097   | 0,8203   |
| u(PV)           | 0,0617      |              |          |          |             |          |           |          |          |          |
| U(PV)           | 0,1234      | <del>-</del> |          |          |             |          |           |          |          |          |

Figure 3 shows relative contribution of each uncertainty parameter from the model equation. It can be clearly seen that the biggest impact on  $u_M = u_{PV}$  has the repeatability (data derived from method validation).

Combining these data for  $u_{M}$ ;  $u_{PV}/PV = 0.0617/9.9971 = 0.6172\%$  with the  $u_{lab}$  data from the IOC 2004 scheme – bias% = 2.919% and adopting the equation

$$u_A = \sqrt{(u_M)^2 + (u_{lab})^2}$$

we calculate the value = 3.045%. After converting the combined standard uncertainty to the expanded uncertainty, U, using coverage factor of k=2 and reporting 2 significant digits, the result becomes PV =  $10.00 \pm 0.61$  mmol/kg.

# Second type of evaluation

After the successful participation in different PT schemes and after gathering all the data from control charts and after calculating the intra-laboratory (=within laboratory) standard deviation, we were able to evaluate somehow more complex estimation of the expanded standard uncertainty for the determination of the peroxide value. We used the combination of e) and g). The

labs uncertainty changed as well. We used the bias data not only from a single, but from a few PT schemes we participated in – in all 9. The same goes for the e). Using the guide (Nordest, 2004), robust statistics for the calculation of the robust means and robust standard deviations for each PT scheme – algorithm A (ISO/DIS 13528, 2002), we were able to calculate all the values and set the following Table 2.

After calculating the intra-laboratory (=within laboratory) standard deviation  $S_{Rw}$  from control charts with the aid of (ISO 5725-2, 1994), which was 6.039451 %, we had all the necessary data for the uncertainty calculation. We had to calculate the "bias" uncertainty  $u_{bias}$  from laboratory bias

$$\left(RMS_{bias} = \sqrt{\frac{\sum bias_i^2}{n}}\right)$$

and the method bias (C<sub>ref</sub>) using equation

$$u_{bias} = \sqrt{RMS_{bias}^2 + u(C_{ref})^2} .$$

We calculated the method bias from the pooled robust relative standard deviations from the schemes and the laboratory bias from the Bias% data from Table 2.

Tab. 2: Robust statistics data for samples from different PT schemes. Legend:  $X_{ref}$  = assigned value;  $X_{lab}$  = our labs value; Bias% = relative percentage difference between  $X_{lab}$  and  $X_{ref}$ ; Szv% = relative robust standard deviation of the set; n labs = number of labs participating.

Tab. 2: Podatki robustne statistike za vzorce iz različnih shem PT. Legenda:  $X_{ref}$  = asignirana vrednost;  $X_{lab}$  = podatek našega laboratorija; Bias% = relativni odstotni odmik med  $X_{lab}$  in  $X_{ref}$ ; Szv% = relativni robustni standardni odmik niza; n labs = število sodelujočih laboratorijev.

| Year | Sample    | Xref | Xlab | Bias % | Szv % | No. labs |
|------|-----------|------|------|--------|-------|----------|
| 2003 | coi 23-03 | 5.31 | 5.46 | 2.92   | 7.72  | 48       |
| 2004 | coi 24-04 | 1.65 | 0.99 | -40.30 | 28.06 | 48       |
| 2004 | coi 25-04 | 4.68 | 4.01 | -14.23 | 10.50 | 48       |
| 2005 | coi 28-05 | 6.34 | 6.23 | -1.74  | 7.45  | 49       |
| 2005 | coi 29-05 | 4.54 | 4.21 | -7.27  | 7.45  | 49       |
| 2004 | B_21_360  | 2.05 | 2.15 | 4.88   | 24.39 | 8        |
| 2004 | B_21_358  | 0.80 | 1.25 | 56.25  | 75.00 | 6        |
| 2004 | B_21_359  | 3.65 | 4.15 | 13.70  | 52.06 | 5        |
| 2005 | B_21_364  | 4.45 | 4.85 | 8.99   | 29.21 | 8        |

Finally we combined the bias uncertainty data with intra-laboratory standard deviation  $S_{\text{Rw}}$  data using equation

$$u_{PV} = \sqrt{u_{bias}^2 + s_{Rw}^2} \ .$$

In this way calculated combined uncertainty was quite elevated, so we had a detailed look at the data. After inspecting the PV ranges, we decided to split the data and uncertainty evaluation in two different concentration ranges: from 0–2 mmol/kg and from 2–10 mmol/kg. The 2 to 10 mmol/kg range has the calculated expanded uncertainty U with the coverage factor of k=2 for the value 9.9971 of 1.8 mmol/kg, so the result reads  $PV = 10.0 \pm 1.8 \text{ mmol/kg}$ .

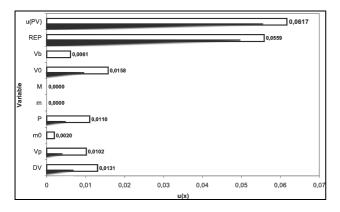


Fig. 3: Relative contribution of each uncertainty parameter from the model equation.

Sl. 3: Relativni prispevek posameznih segmentov negotovosti.

# **CONCLUSIONS**

Considering both types of evaluation, it can easily be concluded that the first evaluation has much smaller U when compared to the second one. It is obvious that the partial components from the uncertainty budget have very limited influence compared to the repeatability influence, which is again relatively small compared to the laboratory (bias) influence from the PT scheme(s). On the other hand, of a real value are only repeatability (within-laboratory reproducibility) data from many years' control charts and results from PT schemes - in the lack of CRM's – giving us data for the method and laboratory uncertainty. So, if the lab wants to have relatively small U, it will have to emphasize its repeatability demands on their control charts, participate in good PT schemes and it will have to be able to afford itself to ignore certain schemes' results (the ones where the method uncertainties are relatively big compared to its own proficiencies). This is the only way to real, realistic and relatively small measurement uncertainties.

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# OVREDNOTENJE MERILNE NEGOTOVOSTI ANALIZNIH METOD ZA UGOTAVLJANJE ZNAČILNOSTI OLJČNEGA OLJA IN OLJA IZ OLJČNIH TROPIN IZ UREDB EGS 2568/91 IN ES 1989/2003 – PRIMER OVREDNOTENJA MERILNE NEGOTOVOSTI PEROKSIDNEGA ŠTEVILA

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#### **POVZETEK**

Pred vsako meritvijo moramo biti prepričani, ali vemo, v kateri del stvarnosti, v katero področje fizičnega sveta, sodi. Zdaj postaja samoumevno, da tvori znanstveno disciplino, ki ji pravimo meroslovje. Meroslovje je v naši (znanstveni) zavesti že dlje časa, a ga kemiki nikoli nismo jemali dovolj resno, da bi se pri svojih rezultatih, kaj šele pri meritvah, z njim zares ukvarjali. Meroslovje je del znanstvenega procesa v vseh sferah znanosti in zatorej tudi kemije. Kemijsko merjenje je torej primerjava količine merjenca (npr. alfa-tokoferol v oljčnem olju) in njegova naveza na enoto (npr. mol/kg, mg/kg). Pri podajanju rezultata merjenja nastane problem sledljivosti – kako smo lahko zares prepričani, da je rezultat našega merjenja primerljiv z rezultati drugih, ki merijo isto veličino, in seveda obrnjeno. Na koncu uganke in po obdelavi drugega velikega vpliva na kakovost naše meritve (in rezultata) – validacije – leži ovrednotenje merilne negotovosti (OMN). MN je lastnost rezultata merjenja, ki določa, znotraj katerih meja okrog izmerjene veličine se skriva pravi rezultat (in s kakšno verjetnostjo). Dokument EGS 2568/91 s svojimi prilogami podaja analizne metode za ugotavljanje značilnosti oljčnega olja in olja iz oljčnih tropin ter določa meje za posamezne kategorije le-tega. Dokument ES 1989/2003 pa podaja analitsko shemo, algoritem in pot za razlikovanje – kategoriranje posameznih kakovostnih razredov oljčnega olja glede na določene meje. Velik manko evropskih metod je dejstvo, da ni validacijskih parametrov, ki so ključni pri OMN (in pri postopku akreditiranja laboratorija za posamezno metodo).

Več načinov za OMN je: način z uporabo modelne enačbe, z uporabo CRM-jev in s sodelovanjem v shemah PT, ki pa so najbolj problematične – problem asignirane vrednosti. Prava pot navadno leži v trikotniku med vsemi tremi (samo dvema pri večini preskusov pri oljčnih oljih). OMN za posamezno metodo preskušanja je lastna laboratoriju in naj bi rabila za njegovo kakovostno umestitev "na trgu". A žal je v polju preskušanja oljčnih olj pomanjkanje ustreznih CRM-jev zelo pereče. Tako je edina pot, ki jo lahko uberemo do ovrednotenja MN, pot sodelovanja v shemah PT. Mednarodni svet za oljčno olje (IOC) je pomagal pripraviti dokument "Evaluation of the uncertainty of measurements and the critical difference: applications to analytical parameters", katerega avtor je Jose Ramon Garcia Hierro, ki s pomočjo izračunanih/določenih OMN laboratorijem olajša delo. Njihova »edina« skrb je tako zmanjšanje odstopanja od prave vrednosti oziroma povečanje točnosti. Večletne izkušnje našega laboratorija pri sodelovanju v shemah usposobljenosti – IOC, Madrid in Bipea, Francija, so nam pomagale pri postavitvi naših lastnih OMN, ki se nenehno izboljšujejo (problem najšibkejšega člena).

Ključne besede: CRM, EGS 2568/91, ES 1989/2003, metrologija, negotovost, oljčno olje, shema PT

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# MICROSATELLITES AS A POWERFUL TOOL FOR IDENTIFICATION OF OLIVE (*OLEA EUROPAEA* L.) PLANTING MATERIAL IN NURSERIES

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# **ABSTRACT**

Microsatellites were applied to confirm the varietal identity of thirteen olive samples from a private nursery. Allelic profiles of samples were compared to genotyped reference varieties from three olive germplasm collections. Genotyping data of two microsatellite loci (ssrOeUA-DCA3 and ssrOeUA-DCA16) allowed discrimination of ten olive samples and the identification of six olive varieties (Koreneiki, Manaki, Tsounati, Kalamon, Ascolana Tenera, Picual). Mislabelling of two olive accessions (Manzanilla de Sevilla, Coratina) was observed and the lack of reference varieties Nostrana Bitontina and Miloelia hindered the identification of two accessions from the nursery. Accessions denominated as Miloelia and Gaiduroelia were of identical genotype. Different allelic profiles of the Frantoio reference variety were observed from two national olive germplasm collections. Microsatellites were found to be a valuable tool for varietal identity confirmation, in nurseries as well as for screening and managing olive germplasm collections.

Key words: Olea europaea L., microsatellites, identification, variety

# MICROSATELLITI QUALI STRUMENTI APPROPRIATI PER L'IDENTIFICAZIONE DI MATERIALE DA COLTIVAZIONE DI OLIVI (*OLEA EUROPAEA* L.) IN VIVAI

# SINTESI

Microsatelliti sono stati applicati per confermare l'identità delle varietà di tredici campioni d'olivo provenienti da un vivaio privato. I profili allelici sono stati confrontati con le varietà genotipizzate di riferimento derivanti da tre banche genetiche. La genotipizzazione su due locus microsatellitari (ssrOeUA-DCA3 e ssrOeUA-DCA16) ha reso possibile la separazione di dieci campioni di olivo e l'identificazione di sei varietà (Koreneiki, Manaki, Tsounati, Kalamon, Ascolana Tenera, Picual). Gli autori hanno verificato che due dei campioni sono stati contrassegnati erroneamente (Manzanilla de Sevilla, Coratina), mentre causa la mancanza di varietà di riferimento di Nostrana Bitontina e Miloelia non è stato possibile identificare due campioni del vivaio. L'analisi dei campioni delle varietà Miloelia e Gaiduroelia ha evidenziato un genotipo identico. In campioni della varietà di riferimento Frantoio provenienti da due banche nazionali genetiche sono stati riscontrati differenti profili allelici. I microsatelliti si sono rivelati strumenti appropriati per la conferma dell'identità delle varietà provenienti da vivai e per la verifica delle fonti genetiche di olivo provenienti dalle banche.

Parole chiave: Olea europaea L., microsatelliti, identificazione, varietà

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#### **INTRODUCTION**

The olive tree is one of the oldest cultivated fruit species in the Mediterranean Basin and the production of olive oil is of great economic importance for the region. The olive oil industry, nurseries and olive growers are all very interested in an accurate identification system of olive varieties, since a choice of varietal structure significantly contributes to the quality of oil produced. Olive is a vegetatively propagated plant and there are more than 1,000 olive varieties under cultivation, which have originated from selections made by growers over many centuries (Rugini & Baldoni, 2005). The identification of varieties is hindered and uncertain due to the long juvenile stage of the olive tree, the presence of different types and clones and the use of many synonyms and homonyms. Correct identification of olive varieties is important especially in nurseries in which high yielding clones are propagated. To prevent mislabelling of plants, determining the identity of the mother plants is necessary before beginning clone propagation. The management and certification of olive planting material therefore requires the application of a fast and reliable method of identifying cultivated genotypes, particularly at the national and regional levels. The introduction of new methodologies into the olive certification scheme will accelerate and optimise the identification process, by allowing the fingerprinting of each genotype at any developmental stage and independently of environmental factors.

Classical approaches for discriminating among olive varieties are based on phenotypic observation and description of morphological markers. The limitations of these markers, which hamper their use in a reliable identification process, are: strong influence of environmental factors and cultivation conditions, subjective evaluation of markers, and the time required for the analysis. By developing biochemical markers, structural gene products such as isozymes were first successfully applied in varietal identification (Trujillo et al., 1995). More recently, PCR-based techniques have been introduced and the first RAPD markers have been used for the molecular characterization and discrimination of olive varieties (Ganino et al., 2006). AFLPs have also been reported as an applicable marker system in the varietal identification process and these markers seems to be more suitable for genotyping olive varieties (Belaj et al., 2003), but the development of microsatellite markers in olives (Rallo et al., 2000; Sefc et al., 2000; Carriero et al., 2002; Cipriani et al., 2002) has provided an improved approach for varietal identification (Bandelj et al., 2002; Lopes et al., 2004). Compared with other DNA fingerprinting techniques, microsatellites are extremely polymorphic, and they have the potential for providing unique fingerprints for each distinct genotype, a useful means of identifying different varieties.

The aim of this paper was to test the applicability of microsatellite markers in identifying olive trees belonging to a set of Greek, Spanish and Italian olive varieties and to confirm the genetic identity of the mother plants in the nursery for propagation purpose.

# **MATERIALS AND METHODS**

#### Plant material

Thirteen olive samples from Greece (Koreneiki 1, Manaki, Kalamon, Miloeliá, Tsunati, Gaiduroeliá, Koreneiki 2), Spain (Manzanilla de Sevilla, Picual) and Italy (Frantoio Fs-17, Nostrana Bitontina, Coratina, Ascolana Tenera) included in a genotyping analysis were provided by a private nursery. Olive varieties used in the analysis as 'reference' for varietal identification and comparison were obtained from the World Olive Germplasm Bank of Cordoba (Spain), the Plant Protection Institute of Thessaloniki-Agricultural Research Station of Khalkidiki (Greece) and the national olive collection of Strunjan (Slovenia). Olive accessions and reference varieties used in microsatellite analysis are listed in Table 1.

# DNA isolation and amplification of microsatellites

Olive DNA extraction by a modified CTAB method and amplification of microsatellites with fluorescencebased detection was performed as previously reported by Bandelj et al. (2004a). Two primer pairs for olive microsatellite loci ssrOeUA-DCA3 and ssrOeUA-DCA16 (Sefc et al., 2000) were redesigned by adding an universal M13(-21) tail (5'-TGT AAA ACG ACG GCC AGT-3') to their 5' ends (Schuelke, 2000). The third, universal M13(-21) primer was labelled with Cy5, allowing incorporation of fluorescence dye into the PCR fragment and its subsequent detection. Amplification reactions were carried out in a total volume of 10 µl, containing 20 ng genomic DNA, 1X supplied PCR buffer (Promega), 0.2 mM of each dNTP, (Roche), 0.25 unit of Tag DNA polymerase (Promega), 0.5 µM of sequence-specific reverse primer, 0.5 µM of fluorescence labelled universal M13(-21) primer and 0.125 µM of forward primer with M13(-21) tail. Amplification was performed in a GeneAmp 9700 thermal cycler (Applied Biosystems) and the conditions of the two-round PCR amplification were as follows: 94 °C (5 min), then 26 cycles at 94 °C (30 s) / 50 °C (ssrOeUA-DCA3) or 52 °C (ssrOeUA-DCA16) (45 s) / 72 °C (45 s), followed by 8 cycles at 94 °C (30 s) / 53 °C (45 s) / 72 °C (45 s (ssrOeUA-DCA3), 1 min 30 s (ssrOeUA-DCA16)), and a final extension step at 72 °C for 10 min. The PCR products were denaturated by adding an equal volume of formamide loading dye (98% formamide, dextran blue 5 mg/ml) and by heating at 94 °C for 4 min. Amplification products were separated on a 7.5% polyacrylamide denaturing gel, containing 7 M Dunja BANDELJ & Branka JAVORNIK: MICROSATELLITES AS A POWERFUL TOOL FOR IDENTIFICATION OF OLIVE (OLEA EUROPAEA L.) ..., 133–138

urea. Electrophoresis was performed on short glass plates of 80 mm separating length, using an automated ALFexpressII sequencer (Amersham Biosciences). Fluorescence signals were collected every 1 s and stored in a computer. A fluorescence labelled molecular size marker (Cy5 Sizer 50–500; Amersham Biosciences) comprising 10 fragments in the size range of 50 to 500 bp was used as an external size marker. An in-house amplified and labelled PCR fragment from a plasmid

template of the known size (100 or 150 bp) was added to each sample as an internal standard. Allele sizes were determined using the software package ALFwin<sup>TM</sup> Allele Locator 1.01 (Amersham Biosciences).

#### **RESULTS**

To confirm the identity of olive trees that have been chosen as mother plants for propagation of cuttings in a

Tab. 1: List of olive accessions and reference varieties included in genotyping analysis, and observed genotypes (allele sizes in bp) at loci ssrOeUA-DCA3 and ssrOeUA-DCA16. The letters indicate the olive GenBank (S = Standard, Gr = Greece, Sp = Spain, SI = Slovenia). Legend: n = number of amplified alleles;  $n_G = number$  of observed genotypes;  $n_{UG} = number$  of unique genotypes per locus.

Tab. 1: Seznam akcesij oljk in referenčnih sort, vključenih v genotipizacijsko analizo, ter opaženi genotipi (dolžine alelov v bp) na lokusih ssrOeUA-DCA3 in ssrOeUA-DCA16. Črke označujejo gensko banko oljk (S = Standard, Gr = Grčija, Sp = Španija, SI = Slovenija). Legenda: n = Število namnoženih alelov;  $n_G = Število opaženih genotipov$ ;  $n_{UG} = Število edinstvenih genotipov na posameznem lokusu.$ 

| No. | Olive accession / reference variety | Samples and ref. genotypes (Gen<br>Bank and register number) | ssrOeUA-DCA3 | ssrOeUA-DCA16 |
|-----|-------------------------------------|--|--------------|---------------|
| 1   | Koreneiki1                          | *  | 237:237      | 147:151       |
| 2   | Koreneiki2                          | *  | 237:237      | 147:151       |
| 3   | Koreneiki S-Sp                      | Cordoba, R218  | 237:237      | 147:151       |
| 4   | Koreneiki S-Gr                      | Thessaloniki, Greece   | 237:237      | 147:151       |
| 5   | Kalamon                             | *  | 230:251      | 125:127       |
| 6   | Kalamon S-Sp                        | Cordoba, R105  | 230:251      | 125:127       |
| 7   | Kalamon S-Gr                        | Thessaloniki, Greece   | 230:251      | 125:127       |
| 8   | Frantoio Fs-17                      | *  | 230:241      | 151:155       |
| 9   | Frantoio S-Gr                       | Thessaloniki, Greece   | 241:247      | 147:155       |
| 10  | Frantoio S-SI                       | Strunjan, Slovenia   | 235:241      | 151:157       |
| 11  | Manaki                              | *  | 241:243      | 151:155       |
| 12  | Manaki S-Gr                         | Thessaloniki, Greece   | 241:243      | 151:155       |
| 13  | Manzanilla de Sevilla               | *  | 237:247      | 125:176       |
| 14  | Manzanilla de Sevilla S-Gr          | Thessaloniki, Greece   | 243:251      | 155:176       |
| 15  | Manzanilla S-Sp                     | Cordoba, R21   | 243:251      | 155:176       |
| 16  | Picual                              | *  | 237:247      | 127:155       |
| 17  | Picual S-Gr                         | Thessaloniki, Greece   | 237:247      | 127:155       |
| 18  | Picual S-Sp                         | Cordoba, R662  | 237:247      | 127:155       |
| 19  | Ascolana Tenera                     | *  | 230:247      | 127:155       |
| 20  | Ascolana Tenera S-SI                | Strunjan, Slovenia   | 230:247      | 127:155       |
| 21  | Coratina                            | *  | 237:243      | 151:157       |
| 22  | Coratina S-Sp                       | Cordoba, R79   | 237:241      | 151:174       |
| 23  | Tsunati                             | *  | 230:241      | 125:147       |
| 24  | Tsounati S-Gr                       | Thessaloniki, Greece   | 230:241      | 125:147       |
| 25  | Gaiduroelia                         | *  | 247:251      | 155:182       |
| 26  | Gaiduroelia S-Gr                    | Thessaloniki, Greece   | 241:251      | 125:127       |
| 27  | Nostrana Bitontina                  | *  | 230:251      | 155:176       |
| 28  | Miloelia                            | *  | 247:251      | 155:182       |
|     |                                     | n  | 7            | 9             |
|     |                                     | n <sub>G</sub>   | 13           | 11            |
| •   | _                                   | $n_{UG}$   | 5            | 3             |

<sup>\*</sup> Samples for identification

private nursery, two published loci, ssrOeUA-DCA3 and ssrOeUA-DCA16 (Sefc et al., 2000), were chosen. Previous studies (Bandelj et al., 2004b; Lopes et al., 2004) have shown that these two microsatellite loci are extremely powerful in genotyping olive varieties, due to their high polymorphic information content (PIC value), low probability of identity (PI value) and high effective number of alleles. Microsatellites were successfully amplified, revealing a total of sixteen alleles in all twenty-eight olive samples. Allele sizes were accurately determined with the help of a fluorescent labelled molecular size marker as an external standard and by identification of an internal standard. The size range of the detected alleles was similar to the range reported by Lopes et al. (2004) and La Mantia et al. (2005).

The number of observed genotypes at locus ssrOeUA-DCA3 was 13, while 11 genotypes were found at locus ssrOeUA-DCA16 (Tab. 1). Comparison of DNA profiles of reference varieties from Greece, Spain and Slovenia was performed and identical genotypes of the same variety from Greece or Spain were observed in four olive varieties (Koreneiki, Kalamon, Manzanilla de Sevilla, Picual). Reference variety Frantoio from Slovenia and Greece showed different allelic patterns at two loci, which could be only explained as mislabelling of plants in the collection, since seven different alleles were found in two accessions at the analysed loci (data not shown). The mislabelling probably occurred in Greece, because the genotyping results of Frantoio variety in Slovenia (Bandelj et al., 2002) and Spain (De la Rosa et al., 2004) showed the same allelic profiles at other loci (ssrOeUA-DCA4 and ssrOeUA-DCA9).

The allelic profiles of the thirteen olive samples from the commercial nursery were then compared with profiles of reference varieties from olive germplasm collections. Genotyping data allowed discrimination of ten olive samples, but the identity of only six olive varieties (Koreneiki, Manaki, Tsounati, Kalamon, Ascolana Tenera, Picual) was confirmed.

Samples Koreneiki 1 and Koreneiki 2 showed the same allelic patterns at both loci, thus indicating genetic homogeneity of the analysed trees. Identical genotypes were also observed in Miloelia and Gaiduroelia samples. These two samples could be the same variety, but they did not match the reference variety Gaiduroelia from Thessaloniki. To confirm their identity, more microsatellite loci should be characterized.

Mislabelling was also found of sample Manzanilla de Sevilla, one of the main Spanish varieties, widely distributed in Andalusia and Catalonia. Previous fingerprinting analyses of this variety by AFLP and RAPD markers have shown the existence of intravarietal polymorphism (Belaj *et al.*, 2004). Cipriani *et al.* (2002) assigned these differences to somatic mutations occurring during the process of olive vegetative propagation or to mislabelling of plants in collections.

Another mislabelling case was observed in the Coratina sample, which was characterised by a unique genotype [237:243] at locus ssrOeUA-DCA3. Two samples, Nostrana Bitontina and Miloelia, could not be identified in this work, owing to the lack of standard varieties in olive germplasm collections. These two varieties are probably known at local level only, or their denomination could be uncertain.

Fs-17, an Italian clone of Frantoio obtained by breeding programme and selected for high productivity and very early oil formation, did not match two standards (Fontanazza *et al.*, 1998) as expected. Three accessions of Frantoio shared only two alleles (241 and 151 bp) of eight detected.

# **DISCUSSION AND CONCLUSIONS**

The need for an accurate discriminating system for olives is often reported in the literature, since the identification of olive varieties is important for management of olive germplasm collections, as well as variety protection and certification of propagated plants in nurseries. The introduction of DNA fingerprinting techniques has provided a high resolution system of discriminating and, compared to other DNA based markers, microsatellites are reported as being particularly attractive for genotyping plants, because the level of polymorphism detected is much higher than that detected with any other molecular marker assay (Jakše et al., 2001). Microsatellite marker systems have been reported to be a valuable tool in genotyping olive varieties. The amplification of three microsatellite loci were sufficient to discriminate among nineteen olive varieties in Slovenia (Bandelj et al., 2002) and the high discriminatory capacity of microsatellite markers has also been reported by Rallo et al. (2000), Cipriani et al. (2002), Lopes et al. (2004) and La Mantia et al. (2005).

The high level of mislabelling plants observed in this work confirmed the need to establish a reliable identification system in olives. The greatest challenges in the varietal identification process are: to establish an effective and low cost method for analyzing plants, and to obtain results that are comparable among different laboratories and countries. The choice of a microsatellite marker system seems to be suitable, since highly informative microsatellite loci have been identified and microsatellite genotyping results are comparable and easily exchangeable among laboratories. The confusion in naming olive varieties, different clonal selections of economically important olive varieties and the presence of vast number of synonyms and homonyms supports and demands the establishment and availability of a worldwide genotyping database of reference olive varieties based on microsatellite data. The allelic profiles of some important olive varieties from national collections published in this work could be used by other research

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groups to check the identity of varieties presented here with varieties of the same name in other locations.

In conclusion, microsatellites could be successfully used for confirmation of the varietal identity of olive trees in nurseries, as well as for screening and managing olive germplasm collections.

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# MIKROSATELITI KOT PRIMERNO ORODJE ZA IDENTIFIKACIJO SADILNEGA MATERIALA OLJK (*OLEA EUROPAEA* L.) V DREVESNICAH

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# **POVZETEK**

Mikrosatelite smo uporabili za potrditev identitete sort trinajstih vzorcev oljk iz zasebne drevesnice. Alelne profile vzorcev smo primerjali z genotipiziranimi referenčnimi sortami iz treh genskih bank. Genotipizacija na dveh mikrosatelitskih lokusih (ssrOeUA-DCA3 in ssrOeUA-DCA16) je omogočila ločitev desetih vzorcev oljk in identifikacijo šestih sort (Koreneiki, Manaki, Tsounati, Kalamon, Ascolana Tenera, Picual). Pri dveh vzorcih (Manzanilla de Sevilla, Coratina) smo ugotovili napačno označitev, nerazpoložljivost referenčnih sort Nostrana Bitontina in Miloelia pa je preprečila identifikacijo dveh vzorcev iz drevesnice. Vzorca sort Miloelia in Gaiduroelia sta imela identičen genotip. Pri referenčni sorti Frantoio smo ugotovili različne alelne profile vzorcev iz dveh nacionalnih kolekcij. Mikrosateliti so se pokazali kot primerno orodje za potrjevanje identitete sort v drevesnicah kot tudi za pregled in upravljanje genskih virov oljk v kolekcijah.

Ključne besede: Olea europaea L., mikrosateliti, identifikacija, sorte

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# KAROTENOIDI V OLJČNEM OLJU

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# IZVI FČFK

Pigmenti v deviškem oljčnem olju izvirajo izključno iz oljk ali pa nastanejo med ekstrakcijo in shranjevanjem olja. Na podlagi sestave pigmentov lahko sklepamo na morebitne potvorbe olja in razlikujemo med olji različnih sort. Karotenoide v oljčnih oljih lahko določamo s spektroskopskimi in kromatografskimi metodami. Karotenoidi so učinkoviti antioksidanti, ki pa lahko v določenih razmerah delujejo tudi kot prooksidanti. So učinkoviti dušilci singletnega kisika, delujejo kot svetlobni filter in odstranjujejo proste radikale. Nekateri karotenoidi so provitamini A, morda pa imajo tudi preventivni učinek pri kardiovaskularnih in rakastih obolenjih.

Ključne besede: oljčno olje, karotenoidi, antioksidanti

# CAROTENOIDI NELL'OLIO D'OLIVA

# SINTESI

I pigmenti nell'olio vergine d'oliva originano esclusivamente dalle olive o vengono a formarsi durante l'estrazione e la conservazione dell'olio. In base alla composizione dei pigmenti si può risalire ad eventuali contraffazioni dell'olio e distinguere fra olii di diversi cultivar. I carotenoidi nell'olio d'oliva vengono determinati con metodi spettroscopici e cromatografici. I carotenoidi sono efficaci antiossidanti, che in condizioni particolari fungono anche da proossidanti. Sono efficaci soffocatori di ossigeno singoletto, fungono da filtro luminoso ed eliminano i radicali liberi. Alcuni carotenoidi sono provitamine A e si suppone abbiamo anche un'azione preventiva su malattie cardivascolari e tumorali.

Parole chiave: olio d'oliva, carotenoidi, antiossidanti

# **UVOD**

Karotenoidi so intenzivno obarvane v lipidih topne spojine. Doslej je bilo izoliranih že prek 600 različnih karotenoidov (Afzal *et al.*, 2004; El-Agamey *et al.*, 2004). Naravne karotenoide so našli v fotosintetskih in nefotosintetskih organizmih: v rastlinah, algah, glivah, bakterijah in živalih (Su *et al.*, 2002). V človeku so jih doslej našli le nekaj (približno 19). To so predvsem karotenoidi, ki jih zaužijemo s hrano, in njihovi metaboliti (ogljikovodiki: likopen, α-karoten in β-karoten ter ksantofili: lutein, astaksantin, kantaksantin, zeaksantin, kriptoksantin) (Su *et al.*, 2002; El-Agamey *et al.*, 2004). Živalska tkiva ne morejo sintetizirati klorofilov in karotenoidov, lahko pa jih živalske celice modificirajo in asimilirajo (Giuffrida *et al.*, 2007).

Karotenoide delimo na karotene, ki so ogljikovodiki, in ksantofile, ki imajo na enem ali obeh koncih molekule hidroksilno in/ali karbonilno skupino. Polienska struktura in druge strukturne lastnosti definirajo lastnosti spojin (npr. redoks potencial) pa tudi lokacijo in orientacijo karotenoidov v lipidnem dvosloju v bioloških okoljih. Karotenoidi v dvosloju lahko spremenijo fluidnost in prepustnost membrane (El-Agamey *et al.*, 2004; Scotter & Castle, 2004).

Eden najpomembnejših karotenoidov v prehrani je β-karoten. Dokazano je bilo, da sodeluje pri imunskem delovanju in varuje celice pred mutacijami in nenormalno rastjo (Afzal, 2004). V naravi se β-karoten lahko pojavlja kot zmes različnih izomerov (*trans*, 7-cis, 9-cis, 11-cis, 13-cis, 15-cis, di-cis). Cis-izomeri β-karotena imajo precej nižjo aktivnost provitamina A kot *trans*-izomer, ne vemo pa še, kako izomerizacija vpliva na antioksidacijsko sposobnost in sposobnost za dušenje singletnega kisika. S stališča prehrane je torej pomembno določiti količino *trans*-izomera β-karotena (Luterotti *et al.*, 2002).

Lutein je derivat  $\alpha$ -karotena z dvema različnima iononskima obročema:  $\beta$ - in  $\epsilon$ -obroč (Subagio & Morita, 2003)

# Biološki pomen karotenoidov

V telesu se nekateri karotenoidi – provitamini A lahko pretvorijo v vitamin A. Najbolj aktiven provitamin A je β-karoten. Približno polovico vitamina A zaužijemo s hrano živalskega izvora, drugo polovico pa s sadjem in zelenjavo pretežno v obliki provitamina A, zlasti β-karotena (Insel *et al.*, 2004).

Splošno znano je, da je vitamin A pomemben za zdrav vid, povprečnemu človeku pa so manj znane njegove druge funkcije: ima pomemben vpliv na rast, imunski sistem, medcelično komunikacijo in celično diferenciacijo, ohranja zdrave kosti, kožo in membrane sluznic (Su *et al.*, 2002; Insel *et al.*, 2004).

Številne študije o vplivu karotenoidov na potek raz-

ličnih bolezni dajejo nasprotujoče si rezultate (Su *et al.*, 2002; Giuffrida *et al.*, 2007). Tako še vedno ni jasno, ali in v katerih okoliščinah karotenoidi ščitijo pred kardiovaskularnimi boleznimi in nekaterimi vrstami raka. Poročajo celo, da lahko uživanje β-karotena pri kadilcih poveča tveganje za pljučnega raka (Su *et al.*, 2002; El-Agamey *et al.*, 2004).

Karotenoidi so lahko močni antioksidanti ali prooksidanti. Njihov antioksidacijski potencial *in vivo* je še precejšnja neznanka.

# METODE DOLOČEVANJA

# Spektroskopske metode

Spektroskopske metode so hitre, preproste in poceni metode za določevanje pigmentov v oljčnem olju. Spekter ekstrakta karotenoidnih pigmentov iz oljčnega olja ima obliko prevladujočega karotenoida luteina, z dvema absorpcijskima maksimumoma med 400 in 500 nm. Za določevanje karotenoidov so izbrali valovno dolžino drugega luteinovega maksimuma (472 nm), saj pri tej valovni dolžini ni interferenc prevladujočega klorofilnega pigmenta feofitina a. Količino karotenoidov izračunamo s pomočjo ekstinkcijskega koeficienta (Mínguez-Mosquera *et al.*, 1991). Lahko pa absorbanco merimo pri 449 nm in karotenoide kvantificiramo s pomočjo umeritvene krivulje, pripravljene z β-karotenom (Caponio *et al.*, 2005; Giuffrida *et al.*, 2007).

# Kromatografske metode

Starejše kromatografske metode temeljijo na kolonski ali tankoplastni kromatografiji. Te metode imajo več pomanjkljivosti. Potrebujemo veliko vzorca, metode so dolgotrajne, ločljivost je slaba, karotenoide pa je težko kvantitativno odstraniti s kromatografske plošče. Pigmente je treba pred kromatografijo ekstrahirati iz olja. Karotenoide lahko identificiramo na podlagi adsorpcijskih lastnosti pri tankoplastni kromatografiji pred umiljenjem in po njem, absorpcijskih spektrov v vidnem območju, absorpcijskh trakov v IR, obstoj funkcionalnih skupin pa potrdimo s fizikalno-kemijskimi metodami. Kvantifikacijo opravimo spektroskopsko (Mínguez-Mosquera *et al.*, 1990).

Uvedba HPLC-metod je prinesla številne prednosti. Metode so preproste, hitre, poceni (to je sicer odvisno od detekcije), občutljivost, specifičnost in preciznost so dobre (Su et al., 2002). Uporaba HPLC-metod omogoča istočasno določitev klorofilov in karotenoidov, metode so uporabne za vse tipe rastlinskih olj (Gandul-Rojas et al., 2000). Nekatere HPLC-metode omogočajo istočasno določanje pigmentov in tokoferolov (Psomiadou & Tsimudou, 1998).

V uporabi so nekatere HPLC-metode z normalno fazo (Psomiadou & Tsimudou, 1998). Uporaba reverzne

faze pa prinaša določene prednosti, kot je boljša stabilnost kolone, ponovljivost retencijskih časov in hitrejše uravnoteženje sistema (Gimeno *et al.*, 2000). Večinoma se uporabljajo C18-kolone (Roca *et al.*, 2003; Luaces *et al.*, 2005). Uporaba bolj hidrofobne C30-stacionarne faze namesto običajne C18-faze omogoča boljše ločevanje hidrofobnih spojin, kot so karotenoidi in klorofili, saj omogoča tudi ločevanje izomerov (Su *et al.*, 2002; Giuffrida *et al.*, 2007).

Detektor UV-VIS za mnoge rutinske namene zagotavlja dovolj dobro občutljivost. Z uporabo detektorja z nizom diod (DAD) lahko istočasno spremljamo tudi spektroskopske podatke, ki so nam v pomoč pri določevanju čistosti pika, včasih pa tudi pri identifikaciji neznanih spojin. Detekcija s spektrometrijo s termičnimi lečami je zelo občutljiva laserska fototermična detekcijska metoda, ki doseže mejo detekcije reda velikosti 100 pg/mL (Su *et al.*, 2002) in omogoča tudi ločevanje *trans*- in *cis*-β-karotenov (Luterotti *et al.*, 2002).

Pred analizo karotenoidov in tokoferolov pogosto opravimo umiljenje vzorca, da odstranimo triacilglicerole in klorofile. Po umiljenju naj bi imele HPLC-metode boljšo specifičnost kot kolorimetrične in fluorimetrične metode (Gimeno *et al.*, 2000).

Zaradi dolge verige konjugiranih dvojnih vezi so karotenoidi zelo reaktivni – nestabilni. Da bi se izognili nezaželenim reakcijam karotenoidov, analize opravljamo v temi ali pri zeleni svetlobi (Mínguez-Mosquera et al., 1990; Gandul-Rojas et al., 2000), s temno steklovino (Gimeno et al., 2000), za zaščito karotenoidov dodamo antioksidante (Gimeno et al., 2000). Čeprav bi bilo zaradi reaktivnosti karotenoidov bolje delati pri nižji temperaturi (Su et al., 2002), pa včasih kromatografijo opravimo pri povišani temperaturi: 30 °C (Luaces et al., 2005) ali celo 45 °C (Gimeno et al., 2000).

# KAROTENOIDI V OLJKAH

Deviško oljčno olje vsebuje le pigmente, ki obstajajo v oljkah, in njihove derivate, ki nastanejo med predelavo oljk in kasneje med skladiščenjem olja. Zato je za sestavo pigmentov v olju odločilna sestava pigmentov v oljkah.

Karotenoidi so v vseh fotosintetskih tkivih povezani s klorofilnimi pigmenti (Psomiadou & Tsimidou, 2001). Kloroplaste s klorofilnimi in karotenoidnimi pigmenti najdemo pretežno v epikarpu, v manjši količini pa tudi v mezokarpu (Roca & Mínguez-Mosquera, 2001). V oljkah ne najdemo tistih derivatov pigmentov, ki so povezani s kislim medijem med pridobivanjem olja (feoforbidi, feofitini, 5,8-furanoid karotenoida mutatoksantin in luteoksantin) (Gandul-Rojas et al., 1999).

# Vpliv zrelosti plodov

Sestava pigmentov v oljkah se med dozorevanjem

precej spreminja, zato na pigmente v oljčnem olju močno vpliva stopnja zrelosti plodov, iz katerih je bilo olje pridobljeno.

Količina klorofilnih pigmentov se med dozorevanjem zmanjšuje, nekoliko počasneje pa se razgrajujejo tudi karotenoidi. Kasneje se začnejo tvoriti fenolni pigmenti antocianini, ki plodove postopno temno obarvajo (Gutiérrez *et al.*, 1999; Gandul-Rojas *et al.*, 2000). Tudi zreli plodovi, ki jim barvo dajejo antocianini, še vedno vsebujejo karotenoide in klorofile. Hitrost razpadanja karotenoidov ni konstantna (Roca & Mínguez-Mosquera, 2001).

# Vpliv sorte

Med posameznimi sortami obstajajo statistično značilne razlike v vsebnosti posameznih pigmentov (Roca & Mínguez-Mosquera, 2001). Nekatere sorte imajo veliko pigmentov (Hojiblanca, Picual), druge pa malo (Arbequina, Blanqueta, Cornicabra) (Gandul-Rojas *et al.*, 2000; Roca & Mínguez-Mosquera, 2001).

V oljkah se kljub visoki vsebnosti maščob v plodovih ksantofili ne zaestrijo, kar kaže na to, da se kloroplasti med dozorevanjem plodov ne pretvarjajo v kromoplaste. Izjema so oljke sorte Arbequina, saj so v njih v nasprotju z drugimi sortami našli fitofluen, zaestrene ksantofile ter  $\alpha$ -karoten in  $\xi$ -karoten (Gandul-Rojas  $et\ al.$ , 1999).

Španska sorta oljk Arbequina je izjemna glede sestave in dinamike pigmentov. V zgodnji fazi dozorevanja v njej poteka sinteza estrov ksantofilov, ki jih v drugih španskih sortah niso identificirali. Da gre res za sintezo karotenoidov, potrjuje tudi obstoj prekurzorjev karotenoidov, kot sta fitofluen in ξ-karoten (Gandul-Rojas *et al.*, 1999). Tudi Roca in Mínguez-Mosquera (2001) sta identificirala estra violaksantina in neoksantina v oljkah Arbequina, v štirih drugih španskih sortah pa ne.

# Spremembe sestave pigmentov v oljkah med skladiščenjem

V regijah, ki so velike pridelovalke oljk, so kapacitete oljarn premajhne, da bi lahko sproti predelali vse oljke, zato obrani plodovi včasih v velikih kupih čakajo na predelavo več tednov. Pod vplivom delovanja lipoksigenaz se razgradijo predvsem klorofilni pigmenti, v manjši meri pa tudi karotenoidi. Omenjeni procesi prvi teden potekajo hitro, kasneje pa se upočasnijo (Mínguez-Mosquera *et al.*, 1990).

# Posamezni karotenoidi

V španskih sortah oljk v karotenoidni frakciji pigmentov prevladuje lutein, ki vedno sestavlja več kot 50% vseh karotenoidov. Med dozorevanjem se koncentracija luteina zmanjšuje, njegov delež pa se povečuje (Roca in Mínguez-Mosquera, 2001).

Drugi najpomembnejši karotenoid je  $\beta$ -karoten, ki je edini izmed oljčnih karotenoidov provitamin A. Med dozorevanjem sta se zmanjševala tako njegova koncentracija kot delež v karotenoidni frakciji pigmentov (Roca & Mínguez-Mosquera, 2001).

Skupna količina vseh minornih ksantofilov je v močno obarvanih sortah oljk enaka ali celo večja kot količina  $\beta$ -karotena (Roca & Mínguez-Mosquera, 2001). Doslej so v španskih sortah oljk identificirali anteraksatin, neoksantin, violaksantin,  $\beta$ -kriptoksantin in lutein epoksid. V Arbequini so našli tudi  $\alpha$ -karoten in  $\xi$ -karoten, fitofluen ter estre ksantofilov anteraksantina, neoksantina in violaksantina (Gandul-Rojas *et al.*, 1999; Roca in Mínguez-Mosquera, 2001).

# KAROTENOIDI V OLJU

Barva olja izvira izključno iz barvil, ki izhajajo iz oljk. Olje je rumeno zelene barve, pri čemer je zeleni odtenek odvisen od sorte in zrelosti plodov (Gandul-Rojas et al., 2000). Spekter olja UV-VIS ima širok pas v območju 375-525 nm (maksimumi pri 410, 450 in 470 nm). Pri 670 nm ima olje še en ožji maksimum (Mínguez-Mosquera et al., 1991).

Deviška oljčna olja vsebujejo le pigmente, ki so obstajali že v plodovih, in njihove derivate, ki nastanejo med predelavo oljk v olje (Gallardo-Guerrero *et al.*, 2005; Giuffrida *et al.*, 2007). Obstoj drugih karotenoidov ali drugačne vsebnosti derivatov klorofilov, kot izhajajo iz ekstrakcijskega procesa, kažejo bodisi na potvorbo oljčnega olja bodisi na neustrezen ekstrakcijski proces (Gandul-Rojas *et al.*, 2000; Roca *et al.*, 2003).

Količina vseh karotenoidov v oljčnem olju je od nič do nekaj mg/kg (Psomiadou & Tsimidou, 2001) ali celo do 100 mg/kg (Cichelli & Pertesana, 2004). Ker plod vsebuje 20–30% maščobe, pigmenti pa so topni v njej, bi pričakovali, da bodo koncentracije pigmentov v olju 3- do 5-krat višje kot v oljkah, vendar ni tako, saj med ekstrakcijo olja izgubimo ~80 % klorofilnih pigmentov in ~40 % karotenoidov. Izguba pigmentov je povezana s kooksidacijo z lipoksigenazno oksidacijo nenasičenih maščobnih kislin. Produkti oksidacije so brezbarvni (Mínguez-Mosquera et al., 1990).

Grška olja so v primerjavi s španskimi in italijanskimi bolj zelene barve, kar se kaže tudi v razmerju med skupnimi klorofilnimi pigmenti in skupnimi karotenoidi (Mínguez-Mosquera *et al.*, 1991), ki je v grških oljih 2 do 11 (Psomiadou & Tsimidou, 2001), v sicilskih (Giuffrida *et al.*, 2007) in španskih (Gandul-Rojas *et al.*, 2000; Roca *et al.*, 2003) oljčnih oljih pa je približno ena.

V karotenoidni frakciji pigmentov v oljčnem olju prevladujeta β-karoten in lutein, našli pa so tudi manjše količine drugih karotenov in ksantofilov (Psomiadou & Tsimidou, 2001). Količina luteina v španskih oljčnih oljih je približno dvakrat večja kot skupna količina minornih karotenoidov (Gandul-Rojas *et al.*, 2000; Roca *et* 

*al.*, 2003). Količina luteina in β-karotena v grških oljih je manjša kot v španskih. To lahko pojasnimo s sortnimi razlikami, delno pa tudi s povsem drugačnim analitskim postopkom (Psomiadou & Tsimidou, 2001).

Med ekstrakcijo olja se sprostijo endogene kisline, ki katalizirajo izomerizacijo ksantofilov s 5,6-epoksidnimi skupinami v ksantofile s 5,8-epoksidnimi (furanoidnimi) skupinami. Violaksantin se izomerizira v luteoksantin in auroksantin, anteraksantin v mutatoksantin, neoksantin pa v neokrom (Mínguez-Mosquera & Gandul-Rojas, 1994; Psomiadou & Tsimidou, 2001; Roca *et al.*, 2003; Gallardo-Guerrero *et al.*, 2005; Giuffrida *et al.*, 2007).

Pri ekstrakciji olja nastanejo tudi *cis*-izomeri (*cis*-β-karoten, *cis*-lutein, *cis*-violaksantin), ki nimajo enake vitaminske aktivnosti kot *trans*-izomeri. To dejstvo je pomembno za določitev vnosa teh mikrohranil s hrano (Luterotti *et al.*, 2002; Giuffrida *et al.*, 2007).

V oljih španske sorte Arbequina in v sicilskih oljih so identificirali tudi estre ksantofilov (ester luteina, β-kriptoksantin in njegov ester, ester neoksantina) (Roca *et al.*, 2003; Giuffrida *et al.*, 2007).

# Vpliv sorte

Različna sestava pigmentov v oljkah različnih sort se seveda zrcali tudi v sestavi pigmentov v oljčnem olju. Na podlagi podatkov o vsebnosti pigmentov lahko razlikujemo med sortami oljk (Cichelli & Pertesana, 2004). Med posameznimi sortami lahko ločujemo na podlagi podatkov o skupni vsebnosti pigmentov ter količinah luteina, violaksantina, luteoksantina in auroksantina (Gandul-Rojas et al., 2000; Roca et al., 2003). Med španskimi sortami oljk se po mnogih parametrih, predvsem po obstoju ksantofilnih estrov, razlikuje Arbequina (Gandul-Rojas et al., 2000; Gallardo-Guerrero et al., 2005).

# Vpliv zrelosti

Zmanjševanje količine pigmentov v oljkah med dozorevanjem se seveda kaže tudi v sestavi pigmentov v olju (Gutiérrez *et al.*, 1999; Gandul-Rojas *et al.*, 2000). Pri nekaterih sortah (Leccino) se količina pigmentov med dozorevanjem oljk bistveno ne spreminja, medtem ko se pri večini sort zmanjšuje. Karotenoidi se razgrajujejo počasneje kot klorofilni pigmenti (Mínguez-Mosquera *et al.*, 1991; Gandul-Rojas *et al.*, 2000).

# Vpliv tehnologije

Olja, pridelana v laboratorijskih oljarnah, lahko vsebujejo več pigmentov kot olja iz industrijskih obratov (Salvador et al., 2001). Vsebnost pigmentov je večja v oljih, pridelanih v kontinuirnih oljarnah, kot v oljih iz klasičnih oljarn s stiskalnicami (Cichelli & Pertesana, 2004; Giuffrida et al., 2007). V oljih, pridelanih v

dvofaznem in v trofaznem sistemu, je količina β-karotena enaka, imajo pa olja iz dvofaznega sistema več biofenolov in so zato prehransko bogatejša (Gimeno *et al.*, 2002).

Nenehno stremljenje k izboljšanju kakovosti oljčnega olja spodbuja iskanje novih tehnologij. Ena novejših tehnologij je segrevanje oljk po obiranju (García et al., 2001; Luaces et al., 2005). Olje iz termično obdelanih oljk je manj grenko, segrevanje pa lahko vpliva tudi na količino pigmentov v olju. Količina karotenoidov in klorofilnih pigmentov se pri 24-urnem segrevanju na 40 °C približno podvoji in z nadaljnjim dvigovanjem temperature še narašča. Pri daljših časih segrevanja se količina pigmentov v olju zmanjša; po 72 urah je že manjša kot v olju iz oljk, ki niso bile termično obdelane (García et al., 2001). Nasprotno pa kratkotrajno segrevanje oljk v vodni kopeli pred stiskanjem ne vpliva na vsebnost pigmentov (Luaces et al., 2005).

Na vsebnost pigmentov vpliva tudi temperatura med ekstrakcijskim postopkom. Pri višji temperaturi predelave je aktivnost lipoksigenez manjša, zato je vsebnost pigmentov v olju večja (Luaces *et al.*, 2005).

## Spremembe pigmentov med shranjevanjem olja

Več avtorjev poroča, da se količina karotenoidov v olju, hranjenem v temi, ne spreminja (Gutiérrez & Fernández, 2002; Psomiadou & Tsimidou, 2002a; Caponio et al., 2005; Gallardo-Guerrero et al., 2005). V nekaterih primerih pa lahko pride do izomerizacije minornih ksantofilov neoksantina, violaksantina in anteraksantina. Čeprav poteka izomerizacija ksantofilov v kislem mediju, niso opazili korelacije med vsebnostjo izomeriziranih ksantofilov ter prostimi kislinami v olju (Gallardo-Guerrero et al., 2005). Delno pa se karotenoidi razgradijo tudi v temi, če je v steklenici zrak. Tudi Pagliarini et al. (2000) so poročali, da se je absorbanca pri 475 in 448 nm, povezana s karotenoidi, v oljih, hranjenih v zaprtih steklenicah v temi pri 20 °C, zmanjševala v skladu s kinetiko psevdo-prvega reda. Spremenila se je tudi barva olja.

Gutiérrez *et al.* (2002) poročajo, da je v oljih, hranjenih pri 30 °C na svetlobi, količina karotenoidov padala. Tudi Caponio *et al.* (2005) poročajo o rahlem zmanjšanju količine karotenoidov na svetlobi že pri temperaturi 15-25 °C. Psomiadou in Tsimidou (1998) po treh mesecih na 22 °C nista zaznali spremembe količine β-karotena, kar sta pripisali zaščitnemu delovanju α-tokoferola.

Razgradnja pigmentov je med poskusom pospešenega staranja olja pri 60 °C vidna tudi s prostim očesom. Sprva so spremembe komaj zaznavne – barva olja postane malo manj živa. Te spremembe povezujejo z razgradnjo karotenoidov. Malo pred koncem indukcijske periode pa se olje nenadoma precej razbarva, ta pojav pa se časovno ujema s spremembo – povečanjem hitrosti razpadanja klorofilnih pigmentov (Hrncirik & Fritsche, 2005).

# **ANTIOKSIDACIJSKE LASTNOSTI**

Močni antioksidanti lahko delujejo tudi kot prooksidanti, ker pri njihovi avtooksidaciji lahko nastanejo reaktivne spojine. Pomembno je, da ocenimo njihovo delovanje v bioloških sistemih. Prooksidacijski učinek lahko sproži pojav klorofila ali težkih kovin. Splošno pravilo je, da spojine pri nizkih koncentracijah delujejo kot prooksidanti, pri koncentracijah nad neko kritično vrednostjo pa kot antioksidanti. Znani pa so tudi obratni primeri (Moure et al., 2001).

Karotenoidi lahko delujejo kot antioksidanti (Fakourelis et al., 1987) ali prooksidanti. Na delovanje vplivajo eksperimentalne razmere in prisotnost tokoferolov. Delovanje karotenoidov v procesih oksidacije je zelo kompleksno, saj se tudi sami lahko oksidirajo (Psomiadou & Tsimidou, 2002a). Različne oksidacije, v katere so večinoma vpleteni singletni kisik 1(O2), hidroperoksidi in peroksiradikali, so glavni vzrok za razpad karotenoidov v hrani. Pri termični razgradnji karotenoidov nastanejo hlapni aromatski ogljikovodiki in prek 70 nehlapnih snovi (Scotter & Castle, 2004). Spojine, ki nastanejo pri termični razgradnji β-karotena in likopena, ne vplivajo na fotooksidacijo olja - na svetlobi ne delujejo niti kot antioksidanti niti kot prooksidanti. Očitno njihove molekule nimajo dovolj konjugiranih dvojnih vezi za dušenje singletnega kisika (Steenson & Min, 2000). Polienska struktura omogoča antioksidacijsko delovanje, pa tudi degradacijo spojin pod vplivom svetlobe in/ali toplote (Steenson & Min, 2000).

V literaturi poročajo o različnih mehanizmih, s katerimi karotenoidi ščitijo oljčno olje pred oksidacijo. Karotenoidi so zelo učinkoviti dušilci singletnega kisika. V določenih okoliščinah morda lahko tudi odstranjujejo proste radikale, lahko pa delujejo zgolj enostavno kot svetlobni filter. Podrobneje so posamezni mehanizmi pojasnjeni v nadaljevanju.

# Dušenje singletnega kisika

Klorofili, ki se pojavljajo v oljčnem olju, so fotosenzitivne snovi in se pod vplivom svetlobe lahko vzbudijo v tripletno stanje. S prenosom energije elektrona z vzbujenega tripletnega stanja klorofila na kisik iz osnovnega tripletnega stanja kisika nastane vzbujeni singletni kisik. Tako se lahko sprožijo poškodbe DNK in peroksidacija lipidov (Edge *et al.*, 1997). Singletni kisik je 1000- do 1500-krat bolj reaktiven kot tripletni kisik, ki je vpleten v avtooksidacijske procese, zato v oljih na svetlobi fotooksidacija poteka hitreje kot avtooksidacija. V takšnih razmerah olje ščitijo predvsem antioksidanti, ki delujejo kot dušilci singletnega kisika (tokoferoli in karotenoidi), vloga antioksidantov, ki odstranjujejo pro-

ste radikale (biofenoli), pa je manj pomembna (Caponio *et al.*, 2005).

S prenosom energije elektrona s singletnega kisika na molekulo karotenoida nastane tripletno stanje karotenoida, ki odda prejeto energijo v obliki toplote in se hitro vrne nazaj v osnovno stanje. Molekule z večjim številom konjugiranih dvojnih vezi imajo nižje energije vzbujenih stanj (S<sub>2</sub>, S<sub>1</sub>, T<sub>1</sub>) in bolj učinkovito dušijo singletni kisik (Edge *et al.*, 1997; Steenson & Min, 2000; Psomiadou & Tsimidou, 2002a).

Vsebnost  $\beta$ -karotena in luteina se med fotooksidacijo skoraj ne spremeni, kar potrjuje hipotezo, da antioksidacijsko delovanje karotenoidov temelji na fizikalnem procesu – hipotezo o dušenju singletnega kisika (Psomiadou & Tsimidou, 2002b).

Izmed naravnih karotenoidov singletni kisik najučinkoviteje duši likopen (Woodall *et al.*, 1997). To pa še ne pomeni, da je likopen tudi boljši antioksidant kot βkaroten, saj se likopen laže oksidira in se tako hitreje porabi (Edge *et al.*, 1997). Dušilec singletnega kisika je tudi β-kriptoksantin (Su *et al.*, 2002).

Na učinkovitost dušenja vpliva tudi topilo. Dušenje je manj učinkovito v bolj viskoznih topilih in v topilih, v katerih pride do agregacije; bolj polarni karotenoidi v nepolarnih topilih agregirajo (Edge *et al.*, 1997). Do agregacije lahko pride, ko je dosežena kritična lokalna koncentracija. O kemiji agregatov ne vemo dosti, na podlagi spektralnih sprememb pa lahko sklepamo, da agregacija vpliva na sistem konjugiranih dvojnih vezi. Agregacija ne poteka pri vseh karotenoidih enako lahko, struktura karotenoida pa vpliva tudi na tip nastalega agregata (El-Agamey *et al.*, 2004).

Glavni mehanizem dušenja je prenos energije elektrona. Možno pa je tudi kemijsko dušenje singletnega kisika, pri katerem pa se karotenoid uniči. Seveda je zato bolje, da fizikalno dušenje poteka v večji meri kot kemijsko (Edge *et al.*, 1997).

Čeprav so karotenoidi zelo dobri dušilci singletnega kisika *in vitro*, pa v bioloških sistemih ni nujno tako.

# Radikalski mehanizem

Radikalski mehanizem antioksidacijskega delovanja karotenoidov še ni dovolj raziskan (Steenson & Min, 2000; Hrncirik & Fritsche, 2005). Zdi se, da imajo karotenoidi pri avtooksidaciji nevtralno (Aparicio *et al.*, 1999) ali pa celo negativno vlogo zaradi oksidiranih produktov, ki lahko reagirajo z lipidnim substratom in tako pospešujejo oksidacijo (Steenson & Min, 2000; Subagio & Morita, 2001).

Karotenoidi lahko odstranjujejo proste radikale na enega ali več izmed naštetih načinov (El-Agamey *et al.*, 2004): s prenosom elektrona, z odcepitvijo alilnega vodika ali z adicijo. Pri prenosu elektrona z molekule karotenoida na peroksilni radikal nastane iz karotenoida kation-radikal (Edge *et al.*, 1997; El-Agamey *et al.*, 2004).

Z odcepitvijo alilnega vodika nastane iz karotenoida nevtralen radikal, peroksilni radikal pa se pretvori v hidroperoksid (El-Agamey et al., 2004). Lahko pa poteče tudi adicija karotenoida in peroksilnega radikala (Miller et al., 1996; Edge et al., 1997; El-Agamey et al., 2004). Poudariti pa moramo, da se pri nobenem mehanizmu 'lihi' elektron, značilen za proste radikale, ne izgubi (Edge et al., 1997).

Na sposobnost za odstranjevaje radikalov vpliva obstoj funkcionalnih skupin na končnih obročih. Obstoj karbonilne skupine na obroču precej zmanjša učinkovitost za odstranjevaje radikalov ABTS'+ (2,2'-azinobis(3-etilbenztiazolin-6-sulfonska kislina). Karbonilna skupina privlači elektrone in zmanjša gostoto neparnega elektrona v skeletu z 11 dvojnimi vezmi. Nasprotno pa obstoj hidroksilne skupine na vsakem obroču v β-kriptoksantinu skoraj nima vpliva na sposobnost za odstranjevanje radikalov ABTS'+. Čim večji je kromofor in čim boljše je prekrivanje orbital v njem, tem učinkoviteje karotenoid odstranjuje radikale ABTS'+ (Miller *et al.*, 1996).

Na antioksidacijske lastnosti karotenoidov pa ne vpliva le sposobnost za odstranjevanje prostih radikalov, temveč tudi reaktivnost nastalih karotenoidnih radikalov (Miller et al., 1996; El-Agamey et al., 2004). Karotenoidni kation-radikali in adukt-radikali so močno resonančno stabilizirani in naj bi bili dokaj nereaktivni. Vstopajo lahko v bimolekularne reakcije, pri katerih nastanejo neradikalski produkti. Lahko pa reagirajo z radikali, ki "napadajo", in tako končajo radikalske reakcije (Miller et al., 1996). Potencialno koristni oziroma škodljivi produkti, ki nastanejo iz karotenoidov, so številni cis-izomeri, produkti oksidacije, predvsem epoksidi, in spojine, nastale z razcepom molekule, predvsem apo-karotenali in apo-karotenoli (El-Agamey et al., 2004).

Na hitrost in mehanizem odstranjevanja prostih radikalov vpliva tudi medij oziroma topilo. Karotenoidi so hidrofobne molekule in jih torej najdemo predvsem v lipofilnih območjih. Če karotenoidna molekula leži v notranjosti membrane, je prvi tip reakcije (prenos elektrona) verjetno termodinamsko neugoden zaradi nepolarnega okolja, ki ne podpira ločitve naboja. Tak primer sta β-karoten in likopen v človeških tkivih. Bolj verjetna pa je taka reakcija pri polarnih karotenoidih, npr. pri karotenoidnih diolih, kot je zeaksantin (El-Agamey *et al.*, 2004).

Na antioksidacijsko aktivnost karotenoidov nedvomno vpliva koncentracija kisika (Edge *et al.,* 1997). Pri visoki koncentraciji kisika se karotenoidni radikali, nastali v reakciji s peroksil radikali, lahko avtooksidirajo ali pa nadaljujejo verižno peroksidacijo lipidov. Pri nizki koncentraciji kisika pa je propagacija oksidacije manj verjetna. Ker je koncentracija kisika v različnih tkivih različna (v pljučih je parcialni tlak kisika ~150 mm Hg, v drugih tkivih pa vsaj 10× manjši), delovanje karote-

noidov v različnih tkivih ni nujno enako. (El-Agamey *et al.*, 2004)

Lipoksigenaze razbarvajo pigmente, vendar je njihovo delovanje posredno – pigmente razbarvajo radikali, ki nastanejo pri lipoksigenazni oksidaciji lipidov. Čeprav v oljkah obstajajo lipoksigenaze, pa druge snovi v plodovih ščitijo pigmente pred oksidacijo. Te snovi bodisi močno zmanjšajo aktivnost lipoksigenaz bodisi hitro porabijo hidroperokside, ki nastanejo pri delovanju lipoksigenaz (Jarén-Galán *et al.*, 1999).

# Filtracija svetlobe

Tretja hipoteza o mehanizmu antioksidacijskega delovanja karotenoidov pravi, da  $\beta$ -karoten v olju deluje kot svetlobni filter in tako ščiti olje pred oksidacijo (Fakourelis *et al.*, 1987).

# Karotenoidi so tudi prooksidanti

V določenih razmerah lahko karotenoidi delujejo tudi kot prooksidanti (Psomiadou & Tsimidou, 2002b), kar so pokazali tudi poskusi s celičnimi kulturami. Te lastnosti so morda povezane z agregacijo karotenoidov. Poleg tega bi morali preučiti tudi interakcije karotenoidov z radikali karotenoidov, ki pri višjih koncentracijah niso zanemarljive (El-Agamey *et al.*, 2004).

β-karoten deluje kot prooksidant pri visokih koncentracijah karotenoida in v 100 % kisiku. β-karoten reagira s peroksil radikali, pri čemer nastane karotenil radikal. Ta lahko v odsotnosti kisika reagira z novim peroksilradikalom in tako konča verižno reakcijo. V prisotnosti kisika pa se lahko verižna reakcija nadaljuje z nastankom β-karoten peroksilnega radikala. Tudi β-karoten se lahko avtooksidira, avtooksidacija je intenzivnejša pri višjem parcialnem tlaku kisika (Edge *et al.*, 1997).

Tako β-karoten kot lutein lahko v temi in na svetlobi delujeta kot prooksidanta. Obstoj tokoferolov lahko zmanjša prooksidacijski učinek karotenoidov. Karotenoidi so na svetlobi in pri povišani temperaturi neobstojni, njihovi razgradni produkti pa lahko pospešujejo oksidacijo lipidov. Zaestrenje hidroksilne skupine pa lahko stabilizira karotenoid pri povišani temperaturi. Zaestren karotenoid zato ni tako močan prooksidant kot prosta oblika karotenoida (Subagio & Morita, 2003).

# Sinergija z drugimi antioksidanti in prooksidanti

Poročajo o sinergijskem delovanju β-karotena in αtokoferola (Edge et al., 1997; Psomiadou & Tsimidou, 2002b). Učinek sinergije verjetno lahko pojasnimo s tem, da α-tokoferol ščiti β-karoten pred avtooksidacijo (Psomiadou & Tsimidou, 2002b), možna pa je tudi obratna razlaga (Edge et al., 1997; Schroeder et al., 2006). Sinergijski učinek tokoferolov in β-karotena oziroma likopena upada z naraščajočo koncentracijo karotenoida, pri koncentraciji karotenoida 1000 ppm pa je učinek celo antagonističen (Schroeder et al., 2006). Karotenoidni kation radikali se lahko regenerirajo tudi z vitaminom C (El-Agamey et al., 2004), karoteni pa lahko regenerirajo izrabljene fenolne antioksidante (Schroeder et al., 2006). Karotenoidi in klorofilni pigmenti vplivajo na obstojnost olja, saj lahko delujejo kot prooksidanti, zlasti ob morebitni prisotnosti kovin, s katerimi delujejo sinergistično (Cichelli & Pertesana, 2004).

V bioloških tkivih karotenoidi niso v homogenem stanju, ampak imamo opravka z interakcijami različnih karotenoidov v membranah. Tako lahko predvidevamo sinergistično delovanje β-karotena, ki ga najdemo znotraj membrane, in zeaksantina, ki z delom molekule sega izven membrane (El-Agamey *et al.*, 2004).

# Delovanje karotenoidov in vivo

Vloga karotenoidov v oksidacijskih reakcijah je predmet mnogih raziskav, zavedati pa se moramo, da so rezultati vsake raziskave odsev eksperimentalnih razmer in jih torej ne moremo brez ustreznega premisleka posplošiti na drugačne razmere, še manj pa na delovanje v bioloških sistemih.

Mesto nahajanja karotenov v heterogenih sistemih ima pomemben vpliv na antioksidacijski učinek. β-karoten in likopen v liposomu najdemo v hidrofobni notranjosti lipidne dvojne plasti. Tu jih lahko napadejo lipidni peroksilni radikali, manj pa so dostopni vodotopnim radikalom. Pomemben faktor za delovanje karotenoidov bi lahko bil tudi parcialni tlak kisika (Schroeder *et al.*, 2006).

Študije *in vitro* so pokazale, da obstaja optimalna koncentracija karotenoidov, ki zagotavlja maksimalno antioksidacijsko delovanje v človeških celicah. Seveda pa rezultatov študij *in vitro* ne smemo posplošiti na razmere *in vivo* (El-Agamey *et al.*, 2004).

# CAROTENOIDS IN OLIVE OILS

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#### **SUMMARY**

The pigments profile of virgin olive oil is determined by the pigments initially present in the fruits and their derivatives formed during the extraction process. The presence of other carotenoids or chlorophyll pigments at levels other than that associated with the extraction process indicate that oil has been adulterated or the extraction process was incorrect. The yellow pigments in olive oil are carotenoids and the green pigments are chlorophyll pigments. The amount of carotenoids in oil depends on variety, maturation index, treating of olive fruits before extraction, extraction process, and oil treating. Carotenes as well as xantophylls were identified in virgin olive oils. Lutein is usually the predominating compound in the carotenoid fraction of pigments, followed by β-carotene. The rest of carotenoid fraction are minor xantophylls, such as 5,6-epoxides, 5,8-epoxides or furanoids, and xantophyll esters. Cis-isomeres are also formed during the extraction process. The vitamin activity of cis-isomeres is lower as the activity of trans-isomeres. Pigment profile of olive oil is a useful tool for variety classification and authenticity determination. Carotenoids can act as antioxidants or prooxidants. Their antioxidant/prooxidant activity depends on concentration, solvent, free radicals structure, presence of other antioxidants, metals and oxygen. The antioxidant activity of carotenoids has not been satisfactorily explained as yet. There are three possible mechanisms of carotenoids antioxidant activity. Carotenoids are singlet oxygen quenchers and free radical scavengers and they act as light filters. Findings about carotenoids activity in vitro should not be translated to activity in biological systems. Carotenoids are an important part of human diet because of their antioxidant activity and some of them are also provitamins A. Carotenoids probably prevent some diseases, such as cardiovascular diseases and certain cancers, but can in certain circumstances increase the risk of disease.

Key words: olive oil, carotenoids, antioxidants

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# DELO NAŠIH ZAVODOV IN DRUŠTEV ATTIVITÀ DEI NOSTRI ISTITUTI E DELLE NOSTRE SOCIETÀ ACTIVITIES BY OUR INSTITUTIONS AND ASSOCIATIONS

DECLARATION, MANIFESTO FOR IMMEDIATE WORLDWIDE SHARK CONSERVATION ACTIONS



We, the shark researchers of the world, are compelled to urge the governments of all nations to take immediate steps to conserve remaining shark populations worldwide. There is a dire and immediate need to raise human awareness globally about the threat to shark populations and to promote their management, before it is too late.

Sharks are one of nature's most obvious indicators regarding the health of marine ecosystems; sharks and related species of rays and skates are vital fixtures within the intricate and varied food webs that cover fully most of our planet's surface. Fossil records indicate a tenure of over 400 million years and hundreds of species at or near the top of tidal estuarine, neritic, benthic and pelagic environments. These often maligned creatures represent this planet's original and primary jawed vertebrates and they need protections/regulations against some of the many impacts of modern humanity; commercial and recreational shark fishing, exhibit exploitations, habitat disruption, contamination and pollution continues to denude and poison the character of our planet, and those that dwell there.

Sharks have much more cause to fear humans than the other way around. Modern human fishing practices, both commercial and recreational, are eradicating many species of sharks while disrupting their respective ecologies. As a consequence of increased commercial and recreational pressures on shark populations worldwide, their numbers are now in serious global decline.

Many species, including blue sharks, oceanic whitetips, shortfin makos, piked dogfish, smooth-hounds, reef sharks and even the whale shark, are heavily exploited. It is estimated that even if all commercial fishing were to cease, many of the large sharks may not

recover within 50 years, if ever. Ironically, even with shark populations plummeting in both number and former range, they are still being depicted as a hazard to humanity. An estimated 50% of the world shark catch is believed to be taken accidentally while fishing for other species such as tuna and swordfish. This unplanned capture of marine animals is called "bycatch". Pelagic longlines, which are single-stranded fishing lines 18 to 72 kilometres long, with an average of 1500 baited hooks, as well as open ocean drift gill nets (often illegal) literally filter marine life from the seas. In some regions, the number of sharks caught by longline fishermen account for 90% of total captures. As the bony fish fisheries have been depleted, fishermen have compensated by increasing shark captures. However, sharks are often more vulnerable to overfishing than bony fish are due to their slow rate of maturity and low birth numbers.

Having evolved over the past 400 million years at or near to top of the food chain, sharks have developed into creatures with relatively few natural predators. They have thrived despite an arduous reproduction mode consisting of periodic or infrequent copulation followed by long gestation periods whereby they mature slowly and have few young. As apex predators, sharks are not equipped to withstand predation themselves and, for the above reasons, are highly vulnerable to exploitation. Exacerbating the problem is the fact many shark species segregate by size and sex, such that exploitation in a nursery area can be critically devastating. It has been demonstrated that most commercial shark fisheries collapse within a few years due to commercial extinction of the target resource.

Humans catch sharks in order to obtain meat, cartilage, skin, oil and other products. Shark fins are used in Asian cooking to prepare shark fin soups. Recently, the demand for shark fins has increased dramatically, largely due to expanding and developing markets in the People's Republic of China and their competitors in Japan and Taiwan. Shark fins fetch a high price and this has led to the practice of finning sharks at sea, where the fins are sliced off while the rest of the body is discarded overboard. Often, the shark is still alive when finned and will face a slow and agonizing death as it sinks to the seafloor. Almost all species of large and medium-sized sharks are fished for their fins. The gigantic growth industry of shark and shark fin fisheries is no longer relegated to certain Asian cultures but has expanded to markets in Europe, Africa, Central and South America and many developing nations in the Indian and Pacific Oceans. Shark meat and shark byproducts are also increasingly being used as a cheap supplement for livestock and domestic animal feed. Additionally, shark cartilage is fraudulently advertised in pharmaceuticals as serving a role in cancer prevention. This marketing is based on the wrongful assumption that sharks do not suffer from cancer and ignores the mounting scientific research indicating that shark cartilage cannot either cure or prevent this disease. Some shark products, however, are traditional and viable. Shark liver is rich in vitamins and provides oil and squalene, which are used as lubricants, cosmetics and pharmaceuticals. Likewise, shark skin is used as a type of leather and shark corneas have been used as substitutes for human corneas. Teeth, jaws and taxidermied specimens have been used as decorations and as souvenirs.

It is unclear how many sharks are caught annually, but some conservationists estimate the number to be upwards of 100 million. A recent estimate of sharks killed in the fin trade alone stands at 73 million per year. Annual landings of cartilaginous fish reported to the Food and Agriculture Organization (FAO) of the United Nations amounts to around 800,000 tons, but the actual tonnage is likely to be much higher as a result of underreporting. Industrial fishing vessels often operate in flagrant violation of fishing regulations and, in recent decades, it has been estimated that shark populations have declined by over 90%.

Humans also play a role in decreasing shark populations as a result of increased habitat destruction, resource depletion and environmental pollution. Toxic chemicals absorbed or ingested by smaller animals are passed up the food chain through consumption. Top predators, like sharks, are at the highest risk of contamination as toxins accumulate within the food chain, becoming most concentrated at the top.

Several shark species are now protected in some countries, but it is not enough. A comprehensive polynational approach is warranted. Conservation and management of shark fisheries needs to be based upon research in biology, ecology, distribution, abundance and exploitation of sharks, their prey and associated systems.

Despite being important parts of marine ecosystems, shark research is often neglected in favour of the more commercially viable bony fish and collaboration between agencies and academics is often wanting for cooperation. Biological information on the life history of many shark species is necessary to better assess stock status and harvest impact. It is also necessary to better manage fisheries in which sharks constitute a significant level of bycatch. The lack of effective management and policy enforcement in many countries is leading to the

extinction of many shark species. Consequently, the removal of sharks continues to upset and destabilize the ecological balance between predator and prey. The ability of marine ecosystems to support life has been severely crippled and the system is now in danger of collapse.

Therefore, we ask governments of all nations for immediate:

- protection of all endangered shark species;
- total ban on shark finning in national and international waters;
- management of fisheries in which sharks constitute significant bycatch;
- management of directed shark fisheries;
- control of trade and utilization of shark products;
- investment of resources into research on sharks to better assess stock status and harvest impact.

Until this announcement, the petition has been signed by 138 shark researchers.



Shortfin mako sharks (Isurus oxyrinchus) at the Milano fish market, Italy (Photo: A. De Maddalena).

Atlantski makoji (Isurus oxyrinchus) na milanski ribji tržnici (Foto: A. De Maddalena).

Alessandro De Maddalena, Sean Van Sommeran & Wolfgang Leander

OCENE IN POROČILA / RECENSIONI E RELAZIONI / REVIEWS AND REPORTS, 153–154

# OCENE IN POROČILA RECENSIONI E RELAZIONI REVIEWS AND REPORTS

EL MAR DE PIRAN [Ondina Lusa, Kristina Knez e Fulvia Zudič (redazione)] Edizioni Il Trillo, Pirano, 2006

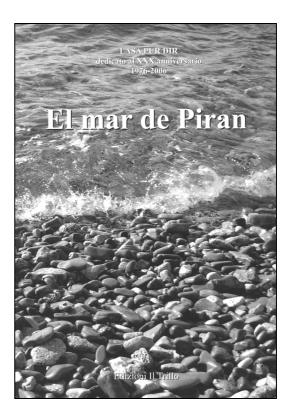
In occasione del trentesimo anniversario del periodico della Comunità degli Italiani di Pirano "Lasa Pur Dir" è stato pubblicato e presentato il volume dal titolo "El mar de Piran". In questo volume viene dato spazio e voce al popolo del mare piranese, gente che ha il mare dentro l'anima e nel presente libro vuole esprimere attimi vissuti, impressi nella memoria collettiva della comunità piranese. Il libro riporta testimonianze legate ad una risorsa naturale, che da sempre ha dato a Pirano ottime risorse di vita, intenso lavoro, guadagno e momenti di vita sana.

Per festeggiare quindi i trent'anni del "Lasa Pur Dir" gli Autori hanno pensato ad un titolo emblematico: El mar de Piran. Questa pubblicazione è dedicata completamente al mare, alla storia di mare, ai suoi sapori, ai suoi personaggi, con anche poesie e termini dialettali piranesi.

Il libro inizia con la narrazione di un personaggio di mare, che forse più di ogni altro personaggio o essere marino rappresenta questo mondo liquido: la figura del pescatore di un tempo.

La sua figura è quella di un uomo semplice, ma quanto mai attuale nei suoi insegnamenti. Egli rispecchia i principi fondamentali che dovrebbero regolare il rapporto uomo-ambiente e la conoscenza della vita che si svolge nel mare. A tale proposito voglio citare una frase del Prof. Giuliano Orel, mio Maestro e piranese di adozione, scritta nella sua presentazione di un libro sul mondo della pesca nell'Alto Adriatico, opera del suo Maestro pescatore Albino Troian, il quale insegnò a lui: "...che il mondo della pesca non è fatto soltanto di uomini, barche, reti, pesci e mercati, ma che queste categorie essenziali non potrebbero raggiungere il loro obiettivo se l'uomo pescatore non fosse anche un ecologo marino. Questo concetto era valido soprattutto in passato per i nostri "vecchi", quando la conoscenza dell'evoluzione del tempo meteorologico, dello stato del mare, delle correnti di marea, delle fasi lunari, delle stagioni, delle migrazioni dei pesci e del loro comportamento potevano costituire la differenza tra una giornata di pesca infruttuosa, irta di rischi per l'equipaggio, l'imbarcazione e gli attrezzi ed una giornata di pesca effettuata in sicurezza con pieno profitto ed in armonia con l'ambiente marino".

Questa citazione custodisce in sé i contenuti principali di "El mar de Piran": la vita di mare, la vita nel mare, le storie di mare, i sapori del mare ... la civiltà del mare. In poche parole, il libro è in grado di trasmettere, con



poco più di un centinaio di pagine, l'anima della cultura e della civiltà istriana ed alto adriatica, in cui Pirano e la sua gente costituiscono uno dei pilastri portanti.

La ricchezza inestimabile della cultura di ieri è la sola base di partenza per un futuro sano ed in equilibrio con la natura ed i suoi tempi e deve essere trasmessa alle generazioni future ed alle comunità di origine continentale che da poco hanno messo radici nel litorale, altrimenti di questo passo, come scrive Ugo Fonda in un capitolo del libro: "forse avremo tanto... ma saremo poco!".

Nicola Bettoso

Tom Turk: POD GLADINO MEDITERANA Založba Modrijan, 2007, 590 str.

O biotski raznovrstnosti Sredozemskega morja še vedno vemo razmeroma malo. Šele v zadnjih desetletjih prihajajo na dan spoznanja, da je biodiverziteta Sredozemskega morja izjemno bogata. In to navzlic velikim spremembam, ki jim je bilo Sredozemsko morje vseskozi izpostavljeno v geološki preteklosti, vštevši tudi nekatera množična izumiranja, ki so za vedno izbrisala dobršen del favne in flore določenega časovnega obdobja. Nova spoznanja so gotovo tudi posledica novejših pristopov, ki omogočajo spremljanje življenja pod morsko gladino.

OCENE IN POROČILA / RECENSIONI E RELAZIONI / REVIEWS AND REPORTS, 153-154

Veliko novih vrst je bilo odkritih, ker so si raziskovalci omislili nove tehnike, povezane z uporabo avtonomne potapljaške opreme. Sicer ni povsem jasno, ali so spoznanja podvodnih fotografov generirala odkritja raziskovalcev ali obratno. A to tudi ni pomembno, gotovo pa je popisovanje, odkrivanje in spremljanje biotske raznovrstnosti tudi domena potapljačev in fotografskih zanesenjakov.

Tudi pričujoče delo *Pod gladino Mediterana* je v prvi vrsti posledica avtorjeve raziskovalne vneme. Tom Turk je potapljaški zanesenjak, odličen fotograf, predvsem pa vrhunski biolog. Ta knjiga ni njegov prvenec, saj je pred tem že objavil knjigo Živalski svet Jadranskega morja. Na 590 straneh avtor predstavlja živalski in rastlinski svet Sredozemskega morja. Najprej nas seznani z osnovnimi abiotskimi in biotskimi značilnostmi Sredozemskega morja, posebno podpoglavje pa posveti tudi življenjskim združbam v slovenskem morju. V tem poglavju avtor posebej piše tudi o prilagoditvah in načinu življenja bentoških živali, zaključi pa ga s podpoglavjem o onesnaževanju Sredozemskega in posebej Jadranskega morja. Nato sledita poglavji s pregledom rastlin in cianobakterij ter pregledom živali v Sredozemskem morju. Prvo poglavje je avtor zaupal Marjanu Richterju, drugo, ki je obenem najobsežnejši del knjige, pa je napisal sam. Za obe poglavji je značilen kratek uvod, temu pa sledi pregled posameznih vrst. Vsaka vrsta je predstavljena z opisom in območjem naselitve ter z ikonami, ki označujejo vrsto dna in globinski razpon, kjer vrsta živi. Ponekod, kjer obstaja možnost zamenjave, so še podatki o podobnih vrstah in o znakih, po katerih vrsti razlikujemo med seboj. Sicer je treba priznati, da najdemo v knjigi kljub vsemu predvsem vrste, ki jih najdemo v Jadranskem morju. Obenem je treba omeniti, kot je napisal že avtor sam v opisu posameznih poglavij, da so v knjigi predstavljene večinoma bentoške alge ter bentoške in nektonske živali, medtem ko je delež planktonskih organizmov zelo majhen. Tako bomo v knjigi zaman iskali fitoplanktonske vrste in holoplanktonske živali, kot so npr. številni raki ceponožci (Copepoda), ščetinočeljustnice (Chaetognatha), repate plaščarje (Appendicularia) in druge. Res pa je, da so predstavljene tiste holoplanktonske živali, ki so najbolj znane in tudi dovolj velike, kot so npr. klobučnjaki.

Avtor se je lotil tudi za moje pojme zelo nehvaležnega dela, to je poimenovanja živali s slovenskimi imeni. Čas bo pokazal, katera od teh se bodo prijela oziroma pri katerih se je avtorju posrečilo skovati primerno ime. Že sedaj se mi zdi nadvse primeren izraz prikrite kozice za vrste iz rodu *Hyppolyte* ali pa rdeči skorjevec za predstavnika grmičastih mahovnjakov *Schizoporella sanguinea*. Gotovo pa so nekatera imena neprimerna, kot je recimo primer školjke sklednice (*Glycimeris* sp.), saj pod tem imenom poznamo več vrst vodnih želv. Podobno velja npr. za podkovnjaka iz rodu *Phoronis*, predstavnika mahovnjakov. Pod imenom podkovnjaki si namreč predstavljamo netopirje iz rodu *Rhinolophus*.



Največjo vrednost knjigi dajejo izvrstne fotografije. Te so poleg avtorja prispevali številni slovenski podvodni fotografi, od katerih je treba zaradi velikega deleža omeniti predvsem Boruta Furlana. Poznavajoč avtorja je bilo pričakovati, da bo v knjigo uvrstil tudi podpoglavje o strupenih živalih, kjer je najbolj doma. Knjigo zaključujejo preglednica o zavarovanih območjih, uporabljeni literaturni vir, slovarček strokovnih izrazov ter indeksi slovenskih in znanstvenih imen. Knjiga je sicer po strokovni vsebini bolj namenjena širšemu krogu uporabnikov, gotovo pa bo našla prostor na polici tudi pri strokovnjakih. Poleg fotografij se po mojem mnenju odlikujejo tudi dovršene in kakovostne tehnične ilustracije ter zemljevidi.

Založba Modrijan je s projektom pričujoče knjige naredila pogumen korak v slovenski naravoslovni publicistiki. Izdala je delo slovenskega avtorja, ki obravnava živelj celotnega Sredozemskega bazena. Lično oblikovana knjiga ponuja obsežen vir informacij od cianobakterij do morskih sesalcev, pregled značilnih biocenoz in izjemno množico kvalitetnih fotografij o flori in favni, zato je smiselno pričakovati, da bo postala del obvezne literature za vsakogar, ki ga resno zanima pogled pod morsko gladino Mediterana.

Po več kot 130 letih, odkar je izjemni hrvaški polihistor Spiridon Brusina pričel vestno popisovati živelj Jadranskega morja, se tako srečujemo s sodobnim, tehnično dovršenim pregledom življenja v zakladnici narave, ki ji pravimo Sredozemsko morje.

**Lovrenc Lipej** 

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